Vol. 10 No. 2

# PLANT PHYSIOLOGY

APRIL, 1935

## KARL RITTER VON GOEBEL

FRANCIS E. LLOYD

(WITH PLATES I AND II)

It is now just two years since KARL VON GOEBEL passed into the beyond, and it is peculiarly fitting that this Society—The American Society of Plant Physiologists—should select him for especial remembrance at this time.[1] While avowedly a plant morphologist, he was in very deed as much a physiologist directing his efforts at the elucidation of plant form. But more than a botanist, he was a man of extraordinary personality. We honor him today for his influence on our science and thought.

Born in 1855 in Billingheim, Baden, his early youth was overshadowed by illness and the circumstances following the death of his father in 1860. Following his destiny fixed upon by a pious mother, he was educated for the Ministry, but when he was doing his duty as a theological student at Tübingen, he took advantage of the presence of HOFMEISTER to pursue the study of Botany. His predilection grew out of his earlier love of nature awakened in him by his environment, especially the Schwabian Alp, which "won all his love" and first aroused in him an appreciation of nature. It is easy to imagine the influence which HOFMEISTER had on the young GOEBEL, but it led to the grave difficulty of disappointment for his mother; and it was only after three years that the die was cast. In 1876, HOFMEISTER becoming ill, GOEBEL went highly recommended to DEBARY in Strassburg and a year later was promoted to the doctorate. Some months were then spent at the Zoological Station at Naples, after which followed his military obligation in Wuertzburg, whither he was drawn by the presence there of SACHS. What a sequence of scientific fathers: HOFMEISTER, DEBARY, SACHS! Developmental morphology, anatomy, physiology under the masters! And their seed fell on good soil. GOEBEL may be said with much truth to have come in the fulness of time—the great

[1] Annual Meeting, Pittsburgh, December, 1934.

foundation of the science had been laid and it was his privilege to build on that foundation. So well did he work that he became assured of rapid success. In 1881 he became First Assistant at Leipzig, in the same year he was called to Strassburg as Professor Extraordinarius, then to the like status at Rostock with the understanding that he should very soon be made Ordinarius. "Here," GOEBEL writes, "I found nothing but an herbarium; no garden, no institute. Salary 2,400 M. The appropriation available 250 M. a year. My 82-year-old predecessor remarked to me, 'My dear GOEBELSCHEN, I have always sent this amount to my daughter. I suppose it will do little harm if we continue to do so?' It cost me much effort to bring into being a modest institute and a small garden, but I learnt much from the experience."

In the winter of 1885–6 after getting these matters started, FRITZ OLTMANNS was called to be GOEBEL'S assistant and *locum tenens* during his first foreign journey to India, Ceylon, and Java. Next year he was called to both Leipzig and Marburg. Choosing Marburg and liking it well, he planned to end his days there. There he undertook the editorship of *Flora,* which furnished a daily stint of work for the rest of his long life, for the task passed from his hands only with his death. A second journey, this time to South America, yielded much adventure and his descriptions of the Venezuelan paramos in the *Schilderungen.*

But his dream of living out his life in Marburg was not to be. In 1891 he went to München where botany had been for a time in a state of lassitude. And it was here that, beginning with his thirty-sixth year, he carried out his great life-work. It was here that we outside of his Fatherland knew him best. Some of us can recall the meager and poorly equipped Institute near the center of the city within a stone's throw of the *Hauptbahnhof*: GIESENHAGEN in a little room off the main laboratory crowded with students, GOEBEL in his own laboratory, not much larger than GIESENHAGEN'S, and a little lean-to glasshouse. A small but richly planted garden furnished an apparently endless supply of material. GOEBEL'S plans for an adequate glasshouse had been frustrated by a congeries of circumstances but with the happier final result that Nymphenburg was chosen by GOEBEL as the site for the Institute and Garden and, with the support of his friend the King, these were brought to fruition and constitute a material memorial to him and his life-work. The Garden was designed to serve not only science but the people. Every day during the growing season sees troops of school children wandering here and there, orderly and under guidance one may be sure, satisfying their rampant curiosity for which there is endless material, the *Biologische Abteilung* and the glasshouses being the chief attractions. For the public in general, an extensive formal garden with a lily pool gives delight. To GOEBEL this Garden was his realm, and to his students it was and is the source of a

KARL RITTER VON GOEBEL
1855–1932

wealth of material of biological significance. The extensive range of glasshouses have collections which in their relative importance and arrangement bear the stamp of the master's thought. He was particularly proud of the treefern house—and a beautiful thing it is,—but scarcely less of the tropical and larger central house. I have the impression that a photograph here reproduced (plate I) taken of him standing on the rough steps of the central rock-mass in this house among his beloved plants, was the one he liked best; but this is only an impression. Plate II shows him seated in his library.

The new Institute was not altogether to his liking. Always impatient of showiness, the *"Prunk"* of the entrance hall frankly bored him. A place for work—this was his object, and here from 1913 on he worked with the same relentless striving that marked his earlier period.

But the material monument is of less importance than that of the mind. Over 200 publications record his observations and thought. This merely quantitative statement is, however, inadequate. Most of his publications were fairly lengthy, a number of them lengthy, and some massive. The *Organography* alone has the stature of an encyclopedia, but without its perfunctory character. It is a mine of information illuminated by a brilliant light of thought. So all-embracing are the contents that, wherever it may end, all future morphology must take account of, if not begin with, the *Organography*.

All this was the product of an almost fanatically restless mind, restless above all in the acquisition of facts from the study of living plants. It was hunger for materials which took him on long and trying journeys into the tropics, into New Zealand, Australia and South America, whence he brought back extensive collections of material. Of his thorough work in the field I am personally aware, since he turned over to me in most generous fashion the whole of his *Utricularia* collections which contained materials in fluid of no less than about forty species. Keen as was his interest in this genus, it was scarcely less so in many others which he must have collected with equal zest. His last lengthy journey, to Java and Sumatra, was undertaken when approaching 70 years of age and while on shipboard he received a shower of congratulatory telegrams. Shortly after, a *Festschrift* containing contributions of his students and friends did much, I imagine, to heal the wounds of the sad period of the world war.

Though made Emeritus in 1931, he had to carry on for yet another year at the age of 77 when his successor, Professor FRITZ VON WETTSTEIN, took over. It was characteristic of him that his penultimate lecture ended the course; he thus avoided the demonstration which would inevitably have occurred. Even after retirement, as had been his habit through the years past, he made each day a tour of the glasshouses, did his editorial work and carried on investigation, and was beginning, even at that age, a study

of the placenta, which started when he cut in two a *Catalpa* fruit with his penknife and asked me what the huge placenta meant biologically.

To speak briefly of GOEBEL'S work is difficult and a general characterization must here suffice. As everyone knows, it covered an extraordinarily wide range of observation on an array of materials which was made available only by his own explorations in most parts of the world. He thus became aware of the exceeding variety of plant form. But he was not content merely to observe in the restricted sense; he felt the urge to explain form in physiological terms, how it comes about and to what end. To this purpose he was constantly experimenting, to be sure in the simplest sort of way: "For an experiment one needs a plant, a flower-pot with earth and a question," the "question" implying brains!

Though he was little given to speculation, his wide and intimate knowledge of plant form led him to a modification of the Darwinian selection theory. He was convinced that the variety of plant form was much greater than the variety of the conditions under which they grow, and saw in these various products many structures which could not be regarded as directly adaptive, but rather indifferent, being neither harmful nor useful. They can arise or disappear without being subject to selection, or they can group themselves and combine to produce members which may enable the plant to become adapted to quite other conditions than the primary ones, and the principle here implied was one of his chief guides in reflecting on the form relations of the plant.

It is also to his merit that be broke away entirely from the formal morphology of which GOETHE was the chief exponent. It was this freedom from prejudice which enabled him, *e.g.*, to lead us to an understanding of that most curious and puzzling group, the Lentibulariaceae, particularly *Utricularia,* acute observation and close reasoning being exemplified in rare manner in his discussion of the evidence available. It was a mark of his reasoning that he insisted on the distinction of objectivities and names. "First of all let it be understood that in nature there are neither 'leaves' nor 'lateral members.' Both ideas are but abstractions of our minds, not just the expression of the facts of observation." Unless we see this as truth, "morphology stiffens into a dead schematism," that is, "if we do not regard the plant to be what it is in reality, a living body whose functions are carried out in the closest possible relations to the outside world." Thus morphology became a physiology: "Morphology is that which we do not yet understand physiologically."

As indicated, his methods of work were of the simplest, and he could be pretty caustic when, for example, one used a microtome when a hand-section would do the work as well or better. I heard him remark in the earlier days of microtome sectioning that anyone who could cut microtome sections regarded himself as a botanist. To be sure,

there was a microtome in his laboratory: "Das ist ganz schön, aber auch dieses muss mit Sinn angewandt werden; eine blosse Hobeltechnik genügt nicht zu morphologischen Schlüssen."

He always insisted on an adequate historic treatment of a subject and he was thoroughly impatient of work done in ignorance of what had gone before. His waste basket was the richer. But he was still more impatient of inaccurate observation. One might differ from him in a matter of explanation, but if one fouled his anchor chain of observation his criticism was a flaming sword.

"Dazu sei nochmals bemerkt, dass es sich nicht um eine *Theorie,* sondern um *Tatsachen* handelt, für deren Richtigkeit ich gerne auch weiterhin die Verantwortung übernehme und von denen sich jeder überzeugen kann, der eine etwas schwierige entwicklungsgeschichtliche Untersuchung auszuführen versteht."

But let it not be thought that he was an overbearing man. On the contrary, he had a placid manner and was kindness itself in his daily relations. His personality was of a rare kind; he walked the earth with a god-like serenity, and indeed his stature, massive head and level eye started a tradition among the natives of Java with whom he came in contact, so that later travellers heard that a god had been there before them. His beard may have been a factor in establishing the tradition, but yet we confess to a sympathetic appreciation of the feeling that in GOEBEL we saw something *übermenschlich.*

While he walked and talked with kings of the earth, he remained quite unspoiled. When his King made him a knight and his students next morning hailed him with an unusual amount of academic applause, he remarked with his peculiar dry humor, that although he had been made a knight he would still come to his lectures afoot. Of course, it could not be otherwise, for his was a simple if extremely intelligent mind. Honors recorded elsewhere were poured in on him but, while appreciating them, he was not puffed up.

Generous, he gave of his time and material to whomsoever he found worthy. If he was found in error he was readily willing to admit it without abatement of his kindness. A brilliant lecturer, given to a rich dry humor, he depended none the less on the things which he discussed, and the students' desks were always loaded with fresh materials for observation while he spoke of them. His lecture room often looked like a horticultural show.

The favorable season during the summer semester permitted excursions and these usually to the nearby Tyrol, when he was the most genial companion. Even then he had an ailing heart, so that he climbed slowly; otherwise his long legs had set a hard pace for some of us. I recall on a beautiful sunny day, on the swelling bosom of one of the high hills sur-

rounding Innsbruck, finding *Botrychium lunaria,* I think it was, searching with him for an hour for the prothallia without success but with much enlightening talk in which BRUCHMANN was not forgotten. Such excursions, usually for two days, spending the night at a country inn, were a full experience for a young botanist of the western world. GOEBEL was the center of interest about which the international group of students played: it was always *"der Geheimrat."*

And it was while on an excursion to his beloved Schwäbische Alb, whither he ever returned, that he fell and received a severe injury from which, had his heart not failed him, he would have recovered. He died on October 9th, 1932, at the ripe age of seventy-eight years, after being only a few months out of harness but still active as President of the Bavarian Academy of Sciences. I cannot but think that his latter years were made happier for him when, during the last International Botanical Congress at Cambridge in the company of Professor L. JOST, after visiting STEPHEN HALE'S rooms in Trinity, we visited the Chapel and there looked in silence at the memorial to the German students of Cambridge who had died fighting for their country.

His only visit to North America was on the occasion of the International Congress of Arts and Sciences, September, 1904, held under the able guidance of Professor HUGH MÜNSTERBURG, and it was then that we had contact with GOEBEL on our own soil. That occasion was made memorable by his presentaton of his paper "The present-day problems of plant morphology," from which brief quotations have been made above. It should be realized that this was a sort of confession of faith, and for its comprehensive values it should be read by every aspiring botanist no matter in which direction his inclinations lie. It is a masterpiece of clear reasoning. At such a gathering of notable scientific men from all parts of the world, memorabilia inevitably emerged; it is appropriate to mention one to this audience in particular. It was the occasion of an informal dinner fittingly enough held in the "Tyrolean Village," even if a synthetic one, at which were seated VON SCHRENCK, JOHN M. COULTER, HUGO DEVRIES, F. O. BOWER, CHARLES B. DAVENPORT, W. G. FARLOW, WILLIAM TRELEASE, and our own guardian spirit, CHARLES R. BARNES. The modest GOEBEL was the dominant figure there.

May his memory as a man live in our hearts and his teachings in our minds. *Forsan et haec olim meminisse juvabit.*

*Note:*—In preparing the above memorial I have had the benefit of the notices by Professor FRITZ VON WETTSTEIN, Prof. F. O. BOWER and Professor G. KARSTEN.

MCGILL UNIVERSITY
MONTREAL, CANADA

# PROOF OF THE PRINCIPLE OF SUMMATION OF CELL E.M.F.'S

H. F. ROSENE

(WITH FIVE FIGURES)

The distribution of electric polarity in the unstimulated, uninjured root was first demonstrated by LUND and KENYON (**6**) in the roots of *Allium cepa, Eichhornia crassipes,* and *Narcissus.* Observations of the electric polarity of a number of other roots have been made by the writer and will be presented in detail in a separate paper. A distinctive feature of the electric polarity common to all the roots so far examined is the occurrence of a characteristic distribution of E.M.F. per unit length of root.

During the measurements of E.M.F. in the roots of different plants, it was observed that when a drop of water was placed around a region of the root between and not at the electrode contacts, the magnitude of total E.M.F. was altered. The water appeared to act as a shunt, changing the IR drop of the length of root over which the potential difference was being measured. This observation, and the fact that water is a necessary part of the environment in which roots can grow, led to the present study which was undertaken to determine the magnitude and direction of change in electric polarity produced by "liquid shunts" around the root.

Since more is known about the electric phenomena of the onion root (*A. cepa*) than about any other, it was selected as the experimental material. Observations made by LUND and KENYON (**6**), MARSH (**8**), and the writer on hundreds of different roots show that the electric polarity of the onion root changes from time to time. The root may manifest a stable potential difference for hours and then a steady increase or decrease in E.M.F. may appear, or it may exhibit rhythmic fluctuations of E.M.F. It has sometimes been observed that if, at a certain time of day, the roots of one bulb from a group of onions which were set at the same time exhibit rhythmic changes in electric polarity, the roots of most of the bulbs in this set will also manifest rhythm; at another time the roots of all the bulbs will exhibit a stable potential difference. From these facts it is obvious that in any analysis of the bioelectric potentials of the root tip the following must be taken into consideration: (*a*) the form of the curve of distribution of E.M.F. per unit length of root tip at a particular instant; (*b*) the variation of the characteristic form of the curve of distribution from instant to instant; (*c*) slow drifts of increase or decrease in potential difference between any two points which may occur; (*d*) occasional rhythmic fluctuations in E.M.F. which may vary in duration and magnitude from time to time. The changes mentioned in (*b*), (*c*), and (*d*) occur when all known external

conditions are maintained constant. They are therefore conditioned by changes within the root itself. Hence in any experiment in which E.M.F. is modified by external conditions, it is necessary to distinguish between the spontaneous changes which are determined by causes of internal origin and the changes produced by altering the external conditions.

In order to determine whether the observed change in total E.M.F. of the root tip produced by surrounding a given region between the electrode contacts with tap water is uniquely characteristic of the system of maintained bioelectric potentials which is a distinguishing feature of electrically polar tissues, experiments were also made on the injury potential of nerve. It is a familiar fact that a potential difference between the cut end and the longitudinal surface of a nerve or muscle may be established and maintained for some time under suitable experimental conditions. It is also a familiar fact that the injury potential varies with the concentration of ions at the electrode contacts; but the writer knows of no work which shows that this E.M.F. may be modified by placing a conducting medium around an uninjured region between and not at the contacts, as will be shown to be the fact in the root.

## Apparatus and method

The apparatus adapted for use in this investigation has been described in a previous paper (**13**). To surround a given region of the root with liquid, a glass cup (diagram M in figure 1) was used, which consisted of a piece of glass tubing, 5 mm. in outside diameter, with a small cover slip cemented on one end to form the bottom of the cup and a capillary tube cemented to a hole at one side at the base, to serve as a delivery tube. The cup was admitted into the moist electrode chamber through the opening *M*, shown in figure 2 of ROSENE and LUND'S paper (**13**). By means of a micromanipulator, the shunt cup (as it will be called) could be raised into position around the root which penetrated through a small hole in the cover glass that formed the bottom of the cup, or the holder with the bulb could be lowered by a different micromanipulator and the root passed through the cup. The liquid was admitted into and withdrawn from the shunt cup through the capillary tube which was connected to a reservoir. The water flow was controlled by the reservoir stopcock and by raising or lowering the rack and pinion stand supporting the reservoir. The apparatus permitted delicate control of all the manipulations and environmental conditions of the root.

The roots were grown in tap water. Those which were selected varied from 50 to 80 mm. in length. All of the roots but one were removed from the onion bulb just before it was placed in the electrode chamber. The experiments were run at room temperature and measurements of electric potential were made by a Compton electrometer.

The general procedure was as follows: The root was placed in the electrode chamber and contacts were made by using the micromanipulators to move the glass claw projections of the electrode cups into position around the root (see fig. 4, 2A and 2B, ROSENE and LUND **13**). The preparation was allowed to rest for a short period and the distribution of potential was then determined by moving the positive electrode (to quadrants) at the tip toward the negative electrode (grounded) near the base, in steps of 1 or 2 mm., making a reading at each step. The positive electrode was then moved away from the root while the empty shunt cup was placed in position (as in diagram M, fig. 1). A horizontal microscope with ocular micrometer was used to determine the position of the contacts on the root and to make the measurements of length. With the shunt cup in position, observations of the electric behavior of the root under constant external conditions were made for a short period and then liquid (tap water or paraffin oil) was run into the shunt cup from the reservoir. The height of the liquid in the cup was determined by the horizontal microscope. With careful manipulation, no leakage of liquid from the cup occurred and mechanical stimulation was reduced to an insignificant minimum. The length of the period during which the liquid was retained in the cup surrounding the root was varied in different experiments, and sometimes in a single experiment. Throughout each experiment readings were made at 15-second or 1-minute intervals.

## Experiments and results

### PROCEDURE 1

#### ALTERNATE ADDITION AND REMOVAL OF WATER FROM SHUNT CUP

Figure 1, B, is the curve of distribution of potential along a root tip which does not exhibit a negative potential in the region 5–10 mm. from the tip. The diagram of the root below curve B is drawn to the same scale as the abscissa and shows the exact position of the shunt cup, *S.C.*, also drawn to scale, as well as the position of the electrodes which are indicated by arrows. In this experiment a shunt cup 2 mm. in height was employed. It surrounded that region of the root which exhibits the highest electropositivity. Curve A shows that each time the segment of the root surrounded by the cup was filled with water, an immediate and abrupt drop in E.M.F. occurred; and each time the water was withdrawn the E.M.F. of the root was abruptly increased. The magnitude of increase of E.M.F. did not quite equal the magnitude of decrease. This was evidently due to a film of water which remained on the root and acted as a partial shunt when the water was withdrawn from the cup.

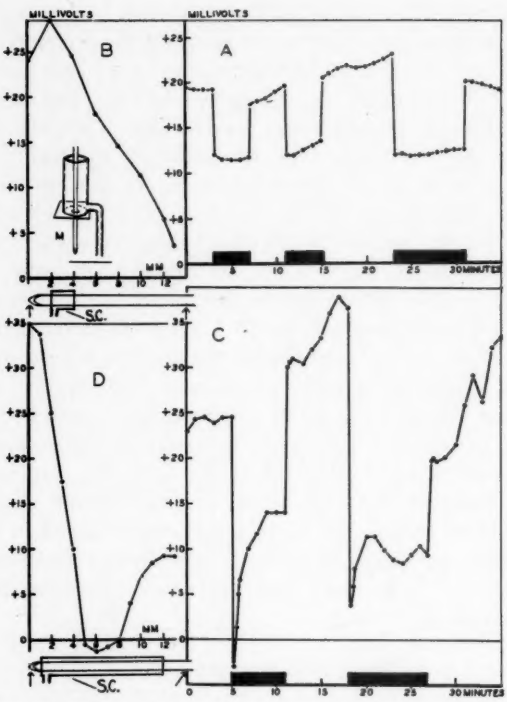


FIG. 1. Effect of liquid shunt around a segment of the root between the electrode contacts on the observed E.M.F. of the root. B and D are the curves of distribution of E.M.F. of two different roots obtained by moving in 2 mm. steps, the positive electrode from the tip toward the negative electrode which was stationary at 14 mm. from the tip. Abscissa gives the position of the positive electrode in mm. from the tip. The diagram below each curve, drawn to the same scale, shows the exact position of the shunt cup (*S.C.*) and electrode contacts (designated by arrows) during each experiment. Curves A and C show changes in E.M.F. when tap water is alternately added to and removed from the shunt cups in B and D respectively. Heavy portions on base lines A and C indicate duration of period when cup is full of water; intervals between when it is empty. Inset M, below curve B, shows shunt cup in position around the root. See text for description.

The height of the larger cup, which was used in the next experiment, was 11 mm. Its exact position on the root is shown in the diagram below curve D, which gives the distribution of potential of that root. The shunt cup (*S.C.*) covered most of the region between the electrode contacts. As indicated by curve C, as soon as water rose upward from the bottom of the shunt cup, there was a sharp decrease in E.M.F. and the electric polarity

of the root was suddenly inverted. During the period when the jacket was filled with water, the E.M.F. increased and normal orientation of the electric polarity was restored; but the potential difference was maintained at a low level. Removal of water produced a sharp rise in potential which reached a new high level. The potential difference continued to increase during the period that the cup was empty. Repeating the procedure produced a similar change in the magnitude of E.M.F., but the electric polarity of the root was not inverted. Similar slow fluctuations of E.M.F. occurred when the shunt cup was filled with water and when it was empty. The abrupt changes in E.M.F. produced by the addition and removal of water from the jacket are readily distinguished from the rhythmic fluctuations of E.M.F., which are associated with changes in the internal processes of the root, by the fact that the abrupt changes in the first case produce an immediate rise in the curve and in the latter case a definite slope is evident. A comparison of curves A and C shows that a greater magnitude of change in E.M.F. was produced in the second experiment. This is explained by the difference in the length of the "liquid shunt" in the two instances, which corresponds to the difference in the height of the water column of the different cups, and also in part by the fact that the total E.M.F. of the root region (14 mm. long) between the electrode contacts was greater in C.

### Procedure 2

#### Addition and removal of water from the shunt cup in steps at definite intervals

When the distribution of E.M.F. was a single unidirectional gradient of fall of potential along the root, as indicated by figure 2, curve B, and water was added to the shunt cup at definite intervals, thus increasing the height of the water column around the root in steps, the E.M.F. of the root was correspondingly altered in steps. This fact is illustrated by curve A, figure 2. Each arrow which points downward indicates that the height of the water column in the cup was raised 1.5 mm., and each arrow which points upward indicates that the height of the water column was lowered 1.5 mm. The positions of electrode contacts and the shunt cup on the root are illustrated by the root diagram B′ below the curves of distribution, figure 2. As shown by curve A, the E.M.F. was diminished each time the water level in the cup was raised by adding more water and it was increased each time the water level of the cup was lowered by withdrawing water. In other words, as the length of the liquid shunt was increased or decreased, the E.M.F. was correspondingly increased and decreased. After the cup had been filled with water, the observed electric polarity of the root re-

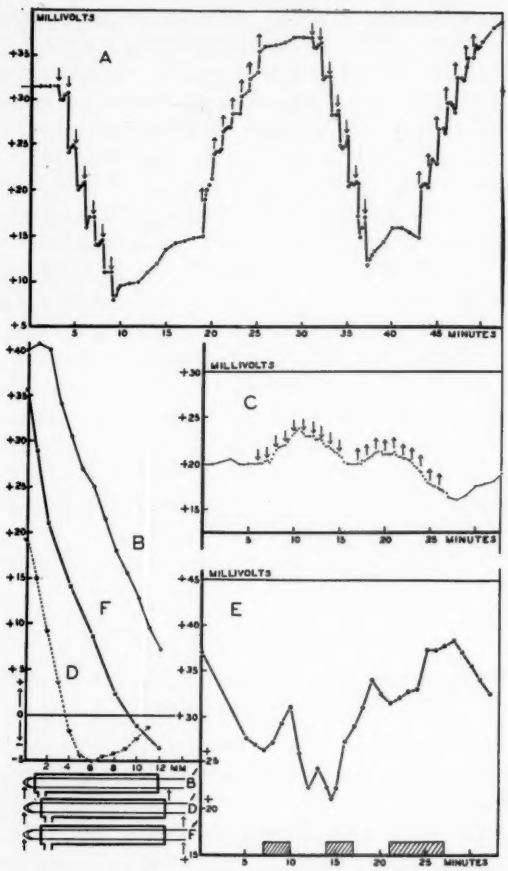


FIG. 2. (*a*) Effect on E.M.F. of root when the liquid shunt is increased and decreased by increments, at successive intervals, and (*b*) absence of effect when paraffin oil is added to and removed from shunt cup. The curves are from three different experiments on three different roots. Arrows pointing downward in curve A show when height of water column in shunt cup was increased by 1.5 mm. increments; arrows pointing upward, when it was decreased in a corresponding manner. Arrows pointing downward in curve C show when height of oil column in shunt cup was increased in steps of 1 mm., arrows pointing upward, when it was correspondingly decreased. Shaded intervals on base line in E indicate when shunt cup was filled with paraffin oil; intervals between, when it was empty. B, D, F are curves of distribution of electric potential for each root and correspond to A, C, and E respectively. Diagrams of the roots B′, D′, F′, below the curves of distribution, show exact respective positions of shunt cup and electrode contacts (arrows) in each experiment.

mained at a relatively lower level; and after the cup had been drained it remained at a higher level. The curves which are represented by figures 1, C, and 2, A, were obtained under conditions in which the height of the water column in the full cup was the same in each case, and the length of the region of the root over which the E.M.F. was measured was also the same.

### Procedure 3

#### Addition and removal of paraffin oil from the shunt cup

In order to determine whether the observed change in E.M.F. of the root produced by adding tap water to the shunt cup could also be produced by adding a non-conducting liquid, paraffin oil was substituted for water.[1] Figure 2, C, shows the results obtained when procedure 2 was followed. The arrows pointing downward indicate the time at which the level of paraffin oil in the cup (which was empty at the beginning of the experiment) was raised in steps of 1 mm. The arrows pointing upward indicate the times at which the oil was removed in a corresponding manner. The curve illustrates the fact that the root exhibited rhythmic fluctuations in E.M.F. but did not show the striking change in E.M.F. which was produced when water was added and removed from the cup around the root under similar conditions, as shown by curve A, figure 2. The corresponding curve of distribution of electric potential for this root is given by figure 2, D, and the position of the jacket by diagram D′, below the curve.

Figure 2, E, was obtained when the shunt cup was at alternate intervals filled and drained with paraffin oil. Throughout the experiment, the root manifested a distinct rhythm in electric behavior, but it shows no quick change as a result of adding and withdrawing oil from the cup. This is clearly evident when the curve is compared to curve C in figure 1, which was obtained by a similar experimental procedure using water instead of oil. The perpendicular changes in curve C, figure 1, which express the alteration of E.M.F. when water was added to or removed from the cup, are entirely absent in curve E, figure 2, when tap water is replaced by the non-conducting oil.

### Procedure 4

#### Addition and removal of water from the shunt cup when position of cup around the root is changed

Figure 3, B, shows the distribution of potential over 25 mm. of a root tip which exhibits two regions with opposed electric polarities. Figure 3, A, shows the curve obtained when the cup was placed, in turn, in two dif-

[1] The paraffin oil was non-toxic, since, when the roots were replaced in water, they grew in a normal manner.

ferent positions corresponding to the regions of opposed polarities and was alternately filled and drained at intervals while in each position. The first part (below the bracket (*a*) of curve A, figure 3) was obtained when the cup was near the apical contact, as illustrated in the upper root diagram B′ below the curves of distribution, and the second part of the curve (below the bracket *b*) was obtained when the jacket was in a position near the basal

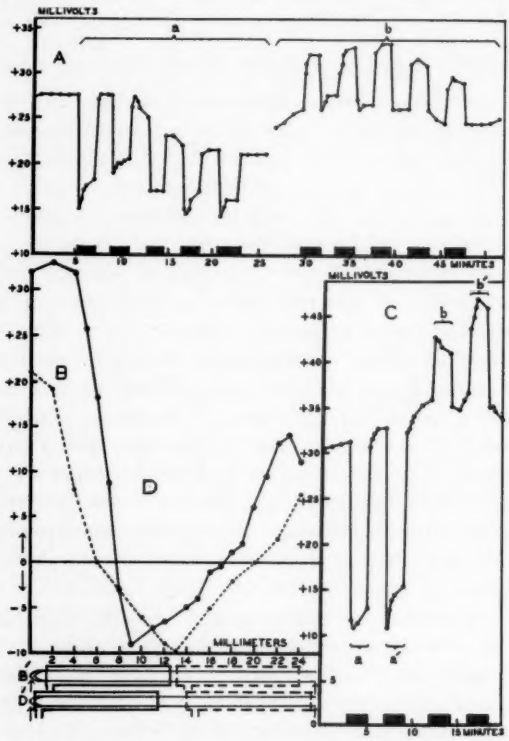


FIG. 3. Effect of placing a liquid shunt on different regions of opposed electric polarities. Curves B and D show distribution of potential in two different roots. Upper root (diagram B′) below the distribution curves shows exact positions of electrode contacts (arrows) and two successive positions of shunt cup in experiments from which curves (*a*) and (*b*) in A were obtained. Lower root (diagram D′) is corresponding diagram for curves in C. In A and C the portions of the curves indicated by the brackets (*a, a′*) were obtained when the shunt cup was in the position indicated by the continuous heavy outline of the cup on each root diagram; those portions indicated by brackets (*b, b′*) when it was in the position indicated by the interrupted outline of the cup. Heavy portions of the base line of A and C show when the cup was filled with water; intervals between, when it was empty.

contact, as indicated by the dotted outline on the same root diagram. In each position the shunt cup was alternately filled and drained at 2-minute intervals. That portion of the curve under bracket (*a*) shows a *decrease* of the total E.M.F. of the root each time water was added to the cup, and an increase when it was removed. On the other hand, that portion of the curve under bracket (*b*) shows an *increase* in E.M.F. each time water was added to the cup and a decrease when it was removed. *The opposite effects on the electric polarity of the root with the cup in the positions corresponding to the regions of opposed polarities, show that the direction of change of E.M.F. produced by adding a liquid shunt is determined by the orientation of the electric polarity in the cells of the shunted region and also demonstrates that the two systems of opposed E.M.F.'s summate in an algebraic manner.*

In another root, following a similar procedure, the shunt cup was placed first at the apical contact and second at the basal contact, so that when it was filled, the entire length of root covered by the cup and the specific electrode contact near it were surrounded by water. In each case therefore a relatively extensive region at the electrode contact was shunted. The results are shown by figure 3, C; the curve of distribution of E.M.F. per unit length of root is shown by figure 3, D; and the positions of the cup and electrode contacts are indicated in the lower diagram of the root D′ below the distribution curves. In each position the cup was filled and drained at 2-minute intervals. As shown by the brackets (*a*, *a′* and *b*, *b′*) in curve C, opposite changes in the electric polarity are observed in apical and basal ends of the root. The greater magnitude of the change shown by the positions of the curves (*a*, *a′*) occurred in the region of the root which exhibited the higher difference in potential.

In a third root, which also manifested the characteristic distribution of E.M.F. with a "valley" of negativity at 8–30 mm. from the apex, as indicated in figure 4, B, the cup was at successive intervals placed in three different positions on the root. It was then filled and drained once while in each position. The successive positions of the cup on the root are indicated below the curve of distribution of E.M.F. by diagrams a, a′, and b. Those portions of curve A, figure 4, which are indicated by the brackets (*a*, *a′*, and *b*), show the corresponding changes in electric polarity of the root when the cup was filled in each of the three positions. The greatest change in E.M.F. occurred when the region with the highest potential was surrounded by water. As in the preceding two experiments, the direction of change of E.M.F. was determined by the orientation of the electric polarity in the region upon which the shunt was applied.

These experiments indicate that if observations are made of the E.M.F. of a given length of root which manifests oppositely oriented polarity poten-

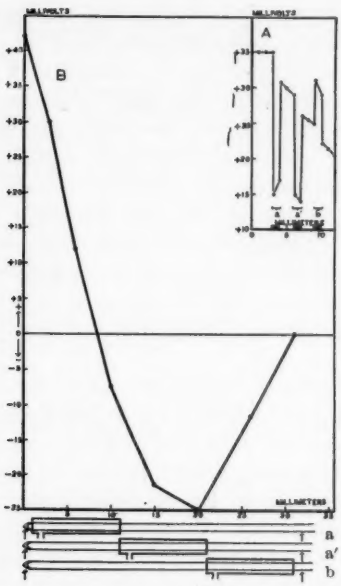


FIG. 4. Effect on electric potential, of addition and removal of a liquid shunt at three different positions on the root with a curve of distribution of E.M.F. as shown in B. Diagrams a, a′, and b below base line show successive and exact positions of shunt cup on same root. Arrows indicate positions of electrode contacts. The parts of the curve above the brackets (*a*) and (*a′*) and (*b*) in A indicate the change in E.M.F. produced by adding and removing water from the shunt cup in the positions corresponding to a, a′, and b of the root diagrams below.

tials, during an interval when the shunt cup filled with water is moved up or down the root, increase or decrease in E.M.F. would appear when the cup passed over a "hill" or "valley" of potential difference. In this way the orientation as well as the relative magnitude of E.M.F.'s per unit length could be determined.

Many such determinations on different roots were made, and the orientation and relative magnitude of E.M.F.'s per unit length was ascertained in this manner. In each case results were checked by comparison with the curve of distribution of E.M.F. per unit length obtained by moving the positive electrode toward the negative electrode as described above. When the empty cup or the cup filled with paraffin oil was similarly moved, no changes in E.M.F. were observed.

These experiments show further that we are dealing with a system or systems of E.M.F.'s, some of which at least have their origin in polar cells arranged in series. This point will be clear if the reader will refer to the

discussion and diagrams of cells in series and in parallel in LUND's paper (**4**) on the theory of cell correlation. In diagram I of figure 2, pages 285, LUND gives a simple hypothetical system of four cells, A, B, C, and D, arranged in series in which A is from the region of highest positive potential, B from a region just proximal to A, C from an isoelectric region, and D from a region of oppositely oriented polarity. When connected in series as illustrated, the total E.M.F. of the system would be equal to the algebraic sum of the E.M.F.'s of the cells between the contacts as represented by circuit 4 in the diagram (fig. 2, I, LUND **4**). If, in such a system, a decrease is produced in the IR drop of cells A or B or both, the total E.M.F. of the system would necessarily fall; and if such a decrease is sufficiently great in magnitude the polarity of the system would be reversed since it would then be determined by the polarity of cell D. This is the explanation for the drop in potential shown in curves A and C, figure 3, and curve A in figure 4 when the conducting medium was placed around a region of high positive potential. If, on the other hand, a decrease is produced in the IR drop of cell D, the E.M.F. of which is opposing the P.D.'s of cells A and B, an increase in the total E.M.F. of the system will appear, as shown by the rise in potential in curves A and C, figure 3, and curve A, figure 4, which occurred when the conducting medium was placed around a region that exhibited a negative polarity potential.

### PROCEDURE 5

#### SHUNT CUP OUTSIDE OF THE ELECTRODE JACKET

Very little or no change in E.M.F. of the root is observed when water is added to or removed from the shunt cup when placed outside of the electrode circuit. Figure 5, X and Y, shows the positions of the electrode contacts in two such experiments. The shunt cup in one experiment was filled with water during the first half of the experiment and with paraffin oil during the latter half. At 1-minute intervals the cup was alternately raised and lowered, thus surrounding 1.5 mm. of the extreme tip with water when raised. The position of the cup when over the tip is indicated by the interrupted outline in figure 5, X. The results are represented by curve A. The stippled portions of the curve show the intervals during which the apex dipped into the water-filled cup and the portions indicated by diagonal lines give the intervals during which the apex dipped into the oil-filled cup. Each time the water-filled cup was raised over the root, a small but definite drop in E.M.F. occurred, and each time it was lowered a small increase in E.M.F. was observed. No such change in E.M.F. was noticed when the oil-filled cup was similarly raised and lowered. In both experiments the effect was very small.

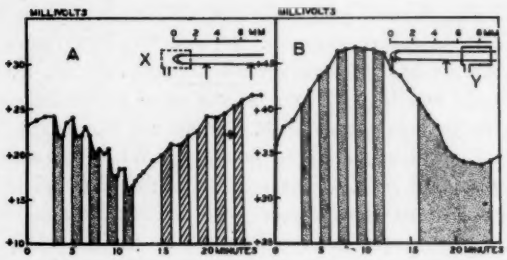


FIG. 5. Effect of tap-water shunt on E.M.F. of a given region when it is applied to a region of the root outside of the electrode circuit. Insets X and Y are diagrams of two different roots which show exact position of the segments of the root covered by the shunt cup, as indicated by the interrupted outline in X and the solid outline of the cup in Y. Stippled areas on the base line of A and B show when the shunt cup is filled with water, diagonal portions when it is filled with paraffin oil, and intervals between when the cup is empty.

In the other experiment the cup was placed in position around a region of the root relatively basal, as illustrated by figure 5, Y. It was filled with water during the intervals represented by the stippled portions of curve B, figure 5. Curve B indicates that no change in E.M.F. is produced by adding and removing water from the cup under these conditions.

Both experiments showed that the electric polarity of the root was in a fluctuating state manifesting rhythm. The small change in E.M.F. observed when the water-filled cup was raised and lowered indicates that part of the electric field at the apical electrode contact was included in the region covered by the cup when it was raised.

### Experiments on frog nerve

The frog's sciatic nerve was carefully isolated and a thread tied around the proximal end by means of which the nerve was gently threaded through the openings of (*a*) the glass projection (contact) of the electrode connection to the quadrants, (*b*) the bottom of the empty shunt cup, (*c*) the glass projection (contact) of the grounded electrode cup, and finally fastened in a glass clamp which thus suspended the nerve in the moist electrode chamber. The electrode cups and glass projections were filled with frog Ringer solution. When both electrode contacts surrounded the uninjured surface of the nerve no potential was exhibited, but when the nerve was cut at the electrode contact (to quadrant) on the distal portion below the cup, an injury E.M.F. appeared. This E.M.F. was not subject to change by adding and removing Ringer solution around the region surrounded by the cup, nor did any effect appear when paraffin oil replaced the Ringer solution. Although the procedures described above in the experiments on the onion

root were repeated with different nerves, the presence of a liquid conductor around the uninjured surface of the nerve between the electrode contacts failed to produce an effect on the injury potential in any way comparable to that which was observed on the maintained E.M.F. of the polar cells in the root tissue. Evidently the systems of E.M.F. are fundamentally different in one or more respects; in the root tissue there is a system of cellular E.M.F.'s arranged in series (and probably in parallel also) such that the addition of a liquid conductor around cells between and not at the contacts will alter the total E.M.F. of that system, whereas in the injured nerve the system is closed and the addition of a liquid conductor between the contacts does not alter the E.M.F. In the latter case, the origins of the P.D.'s are probably limited to the electrode contacts. In the root electrically polar tissue is present, the cells of which are the seats of individual E.M.F.'s that summate algebraically to give the E.M.F. of that system.

## Conclusions

The preceding experiments show that when tap water is placed around a region of the onion root between the electrode contacts the E.M.F. of the root is altered, but when paraffin oil is similarly placed around a region of the root no change in E.M.F. is observed. This fact indicates that when a liquid which contains electrolytes surrounds a region of the root the output of electric energy by the root is altered. The effect on individual cell E.M.F.'s is summated and expressed in the change of the electric polarity of the whole. A liquid shunt around the region of the root which exhibits a positively oriented unidirectional polarity diminishes the electric polarity of the whole, while a liquid shunt around the region which exhibits an oppositely oriented unidirectional polarity increases the electric polarity of the whole. This fact furnishes conclusive evidence that the total observed E.M.F. of the onion root is the algebraic sum of the definitely oriented E.M.F.'s of individual cells.

The fact that the E.M.F. between the cut end and the longitudinal surface of the frog sciatic nerve was not altered by following the procedure used in the experiments on the root shows that the observed effect produced by adding a liquid shunt to the root is uniquely characteristic of the system of continuously maintained bioelectric potentials, distinctive of the root cells.

The fact that the potential difference of the root may be increased or decreased by a liquid shunt indicates that the electric circuit system of the root is not a closed system. In its natural environment the root is exposed to soil or other solutions which contain electrolytes. Bioelectric currents flow outward into the surrounding medium from regions of high electric positivity in the root and currents flow inward from the surroundings to

regions which exhibit relatively low positivity or negativity. Accordingly, the observed phenomena are obviously significant in relation to the problems of (1) transport of ions, (2) absorption of water and solutes by the root, (3) transpiration, and (4) growth.

Energy changes are involved in the process of absorption, and many investigators have shown that permeability and osmotic relations alone are inadequate to explain the phenomena of absorption in the root (**1, 3, 9, 14**). The electric energy produced by the oxidative metabolism of the cells (**5, 13**) is continuously available for work and may be utilized by the root in the processes of absorption and transport. It has been shown that absorption of water and ions by the root is directly correlated with oxidation (**10, 2, 7**), and it has been demonstrated for the first time that the electric potentials of the root and oxygen tension are quantitatively interdependent (**6, 13**). A consideration of the fact that the electric circuit of the root is not a closed system, and the above mentioned relations (*i.e.*, (*a*) of bioelectric currents to oxidation and (*b*) of absorption to oxidation) indicates that these are linked phenomena.

The fact that electric energy is available for the transport of ions, and the additional fact that the E.M.F. of the root is modified only when an electrolytic solution comes in contact with the root, indicate that the available output of electric energy by the root is related to the conductivity of the solutions in which the roots grow. Root growth is dependent upon ion transport and upon the absorption of water and solutes. It may possibly differ in solutions of low and high electric conductivity.

A detailed discussion of the experimental results in relation to the phenomena mentioned above will be omitted, since the purpose of this report is to show that the magnitude and orientation of cellular E.M.F.'s is modified by an electrolytic solution around the root and that the electric circuit of the root is not a closed system, but attention is called to the fact that the study of electric behavior of the root establishes a precise and intelligible approach to the problems of (1) transport of ions, (2) absorption of water and solutes, (3) transpiration, and (4) growth.

## Summary

1. The electric polarity of a given region of the root tip (*Allium cepa*) is decreased or increased when an electrolytic solution such as tap water (liquid shunt) surrounds a segment of that region.

2. The magnitude of change in electric polarity is directly related to the length of the liquid shunt, and the direction of change is determined by the orientation of the polarity potential in the segment to which the shunt is applied.

3. The level of E.M.F. manifested before the liquid shunt is applied is reestablished when the shunt is removed.

4. The observed changes in E.M.F. produced by the addition of a liquid shunt to the root are distinguished from the rhythmic fluctuations in E.M.F., produced by causes of internal origin, by the abrupt change in E.M.F. which occurs when the shunt is added.

5. The observed changes in E.M.F. are determined by the presence of ions in the applied solution. No change in E.M.F. of a given region of the root is observed when a non-conducting liquid is applied to a segment at that region.

6. The results indicate that the system of continuously maintained E.M.F.'s present in the root involves cells arranged in series so that their polar axes coincide.

7. When the liquid shunt is applied to a region outside of the electrode circuit no change in E.M.F. is produced.

8. There is an absence of effect on the injury E.M.F. of frog sciatic nerve following the same procedure used in the experiments on the root. The observed change in E.M.F. of the root, produced by the addition of a liquid shunt, is uniquely characteristic of the system of maintained cellular E.M.F.'s present in the root.

9. The observations furnish direct evidence that the principle of algebraic summation of E.M.F.'s in polar cell systems applies to the electric polarity of the onion root.

The writer wishes to express gratitude to Prof. E. J. LUND for valuable suggestions and criticisms. The investigation was aided by a grant from the research fund of the Department of Zoology, University of Texas.

UNIVERSITY OF TEXAS
AUSTIN, TEXAS

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# EFFECTS OF EXFLORATION ON PLANT METABOLISM

STANLEY AUSTIN

(WITH TEN FIGURES)

## Introduction

The practical importance of the proper balance between the vegetative and reproductive processes in fruit crops has stimulated many horticultural investigations seeking to improve our practical control of these functions. Owing to the slow growth, large stature, and perennial habit of fruit trees as well as their delayed response to experimental manipulation, it has been difficult to secure comprehensive data directly. Consequently considerable work has been done on short-lived annuals, like tomatoes, the development of which is known to be comparable to that of fruit trees.

Use of herbaceous plants in horticultural studies has incidentally provided a considerable amount of agronomic data, and hence it has been natural for the agronomist to extend such investigations in his own field to important herbaceous crops not previously investigated. The existing extensive literature implies that an inverse quantitative relation exists between the reproductive and vegetative processes, growth usually tending to diminish as the size of the fruit crop increases (**4, 5, 7, 13, 14, 21, 22, 23**). It should be noted, however, that structural features of the plant in part determine the relationship between vegetative and reproductive processes. In plants of the determinate habit, the inflorescence utilizes all the apical meristem and hence arrests elongation. According to many investigators, the indeterminate habit, on the other hand, presumably permits stem elongation to continue until the developing fruits begin to monopolize the food supply. The fact that fruiting obviously interferes with elongation in crop plants of the indeterminate growth habit has led to the inference that all plants of this type behave similarly (**26**). It is difficult, however, to see how this interpretation would apply to indeterminate plants which normally set only a small quantity of fruit. Moreover, the rosette vegetative phase of certain long day plants such as spinach suggests that the photoperiod may be as important as reproduction in suppression of stem growth.

In short day plants exhibiting arrested elongation under short day conditions, it is not apparent whether the response is due to fruiting or only to the shorter day length. Although the statement has been made (**2**) that "the reproductive phase of growth apparently curtails the vegetative phase" in the soy bean, a preliminary investigation by the writer dis-

closed that exfloration of the soy bean, an indeterminate short day plant, did not result in increased growth. The present investigation was consequently undertaken to discover why exflorated soy bean plants did not behave like more typical species such as the tomato, which continue vegetative development indefinitely if exflorated.

## Methods

Initial exfloration experiments with soy bean plants grown in the greenhouse seemed to indicate that the vegetative development was not influenced by continued removal of flowers. To determine whether this lack of response to exfloration might have been due to unfavorable conditions in the greenhouse, the experiment was repeated during successive seasons on plants grown out-of-doors. The results being entirely confirmatory, a more intensive investigation was undertaken. Data presented here are for two crops of plants, one grown in the summer of 1931 and another in the summer of 1932. Ito San soy beans were grown out-of-doors in well inoculated soil and all flowers were removed from one series of plants but left to develop on the control series. All plants were watered during periods of dry weather. Material was harvested for chemical analyses at frequent intervals, 20-gm. samples being preserved in alcohol for carbohydrate determinations and larger samples dried in an oven at 100° C. for dry weight, total nitrogen, and ash determinations.

Total organic nitrogen not modified to include nitrates was determined on samples of the dried material. The material preserved in alcohol was extracted several times with 80 per cent. alcohol (**37**), reducing sugars being determined on aliquots of the clarified extract and expressed as dextrose. Aliquots of the same extract were hydrolyzed with hydrochloric acid for the determination of total sugars, which are also expressed as dextrose. The residue from the alcoholic extraction was hydrolyzed by boiling for 2.5 hours with dilute hydrochloric acid under a reflux condenser. Reducing sugars were then determined and expressed as polysaccharides (**20**).

Samples of the dried material were ashed, dissolved in hydrochloric acid, and made up to volume for mineral analyses. Calcium was precipitated as oxalate and titrated with potassium permanganate. Phosphorus was determined colorimetrically by a modification of the Bell-Doisy colorimetric method (**3**). Magnesium was precipitated from the filtrate of the calcium determinations as magnesium ammonium phosphate and the phosphate content of the precipitate determined by the same method as used for phosphorus. Potassium was precipitated as potassium cobaltinitrite and weighed directly in Gooch crucibles.

Material for the 1931 crop was harvested when flowering began and a month later when the fruits had reached almost their full size. The samples designated as "leaves" represent composite samples of all the leaves on the plants. The stems were divided equally into upper and lower halves and are referred to as "upper stems" and "lower stems." Harvesting of the 1932 crop was begun when the plants were still very small and the data are more comprehensive, but unfortunately the plants were badly damaged by winds before the last harvest and their weights are not available. The "tips of stems" represent 2 to 3 inches of the tips of the stems.

FIG. 1. Similarity in appearance of mature exflorated and fruiting soy bean plant.

## Data and discussion

Growth of normal Ito San soy bean plants proceeded rapidly for some time after flowering began, the check in the growth rate seeming to coincide with the enlargement of the fruits. The stem tips of rapidly growing plants were large and blunt, but as the plants reached maturity these tips became more and more slender and the new internodes became shorter until they finally ceased to grow.

Continuous removal of flowers, however, did not increase either height or diameter of the stems or size of the leaves. Growth stopped at the same time in the exflorated and the control plants. There was a slight wrinkling of the leaves of some of the exflorated plants. The similarity in appearance of the exflorated and control plants was striking (fig. 1). The ana-

## TABLE I

ANALYSES OF 1931 ITO SAN SOY BEANS; PERCENTAGES IN TERMS OF DRY WEIGHT

| | JULY 3, 1931 | | | AUGUST 7, 1931 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | BLOOMING | | | EXFLORATED | | | CONTROLS | | | |
| | LEAVES | UPPER STEM | LOWER STEM | LEAVES | UPPER STEM | LOWER STEM | LEAVES | UPPER STEM | LOWER STEM | FRUITS |
| | % | % | % | % | % | % | % | % | % | % |
| Percentage dry weight | 22.20 | 12.15 | 15.09 | 30.80 | 27.71 | 29.03 | 30.90 | 24.77 | 27.15 | 27.05 |
| Total nitrogen | 4.36 | 3.00 | 1.49 | 4.04 | 1.60 | 1.50 | 3.95 | 1.52 | 1.20 | 3.63 |
| Reducing sugars | 1.34 | 2.58 | 2.04 | 1.23 | 2.79 | 1.97 | 1.02 | 4.37 | 4.37 | 2.10 |
| Total sugars | 4.34 | 5.90 | 5.53 | 3.25 | 4.90 | 3.73 | 2.82 | 6.58 | 6.21 | 4.92 |
| Polysaccharides | 17.43 | 15.81 | 16.44 | 17.65 | 22.42 | 26.44 | 12.49 | 16.18 | 18.18 | 21.92 |
| Total carbohydrates | 21.77 | 21.71 | 21.97 | 20.90 | 27.32 | 30.17 | 15.31 | 22.76 | 24.39 | 26.84 |
| Calcium | 1.43 | 1.28 | 1.11 | 1.72 | 1.14 | 0.92 | 1.95 | 0.93 | 0.79 | 0.82 |
| Magnesium | 0.26 | 0.28 | 0.28 | 0.26 | 0.27 | 0.34 | 0.28 | 0.27 | 0.34 | 0.27 |
| Phosphorus | 0.43 | 0.36 | 0.35 | 0.29 | 0.27 | 0.27 | 0.28 | 0.21 | 0.28 | 0.43 |
| Potassium | 2.53 | 4.39 | 3.62 | 1.57 | 1.78 | 1.50 | 1.35 | 1.87 | 1.28 | 2.32 |

lytical data for 1931 are given in percentages of dry weight (table I) and in grams per plant (table II), and are illustrated graphically (figs. 2–5).

TABLE II

SOY BEANS, ABSOLUTE WEIGHTS OF CONSTITUENTS IN GRAMS PER PLANT

| | JULY 3 | AUGUST 7 | | |
|---|---|---|---|---|
| | BLOOMING | EXFLORATED | CONTROLS (STEMS AND LEAVES) | CONTROLS (PLUS FRUITS) |
| | *gm.* | *gm.* | *gm.* | *gm.* |
| Fresh weight | 134.00 | 269.00 | 257.00 | 364.00 |
| Dry weight | 24.00 | 79.50 | 73.00 | 101.80 |
| Nitrogen | 0.795 | 2.23 | 1.94 | 3.03 |
| Reducing sugars | 0.439 | 1.44 | 1.97 | 2.54 |
| Total sugars | 1.21 | 3.01 | 3.36 | 4.79 |
| Polysaccharides | 4.03 | 16.73 | 10.83 | 17.15 |
| Total carbohydrates | 5.24 | 19.74 | 14.19 | 21.94 |
| Calcium | 0.314 | 1.10 | 1.03 | 1.23 |
| Magnesium | 0.065 | 0.222 | 0.212 | 0.295 |
| Phosphorus | 0.096 | 0.222 | 0.190 | 0.326 |
| Potassium | 0.785 | 1.28 | 1.07 | 1.78 |
| C/N ratio | 6.58 | 8.85 | 7.32 | 7.24 |
| $\frac{\text{Moisture}}{\text{Potassium}}$ | 140.20 | 148.00 | 172.00 | 147.40 |

It has been shown by MURNEEK (**23, 25**) that fruit production limits the growth of tomato plants and that defruiting or exfloration greatly increases the size of the plants so treated. Experimental exfloration has no such stimulating effect on the growth of the soy bean. This is a photoperiodic plant in which short day length apparently not only induces flowering but inhibits stem elongation as well. However, a shorter day seems to be necessary to affect the growth rate than to initiate flower formation, because growth continues for some time after flowering begins, long enough in fact to treble the dry weight of the stems and leaves, observations which are also confirmed by the work of other investigators. GARNER and ALLARD (**9**) found that Biloxi soy beans in a ten-hour day stopped growing as soon as flowering began, but under the influence of a 13-hour day they tended toward an everbearing habit so that growth and fruiting proceeded simultaneously. OWEN (**30**) observed that sterile soy bean plants became but little larger than those which set fruits, and that microchemical tests indicated the presence of an abundance of starch in the stems of these sterile plants.

NIGHTINGALE (**27**) believes that in *Salvia* assimilation of nitrogen is limited by a short photoperiod, and as a result carbohydrates accumulate in

the plants which then become fruitful because of the increase in the C/N ratio. Accumulation of carbohydrates in the stems of exflorated soy bean plants (figs. 6, 7) cannot be explained in this way because the soy bean is not limited in its ability to absorb nitrogen under short day conditions. This is evident from the data which show that about 36 per cent. more nitrogen is assimilated by the controls than by the exflorated plants under short day conditions during which fruiting normally occurs. Movement of

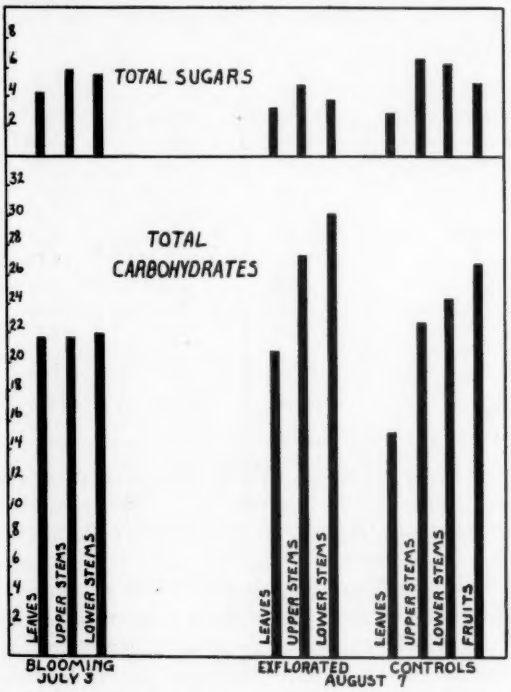


FIG. 2. Composition of soy beans; percentages in terms of dry weight.

nitrogen from the stems and leaves does not account for all of the nitrogen in the fruits. The analytical data not only show that the vegetative reserve is maintained but that more is taken in to supply the demands of the developing fruits. This interpretation agrees with the observation made by WEBSTER (**39**) that nitrogen in the soy bean is not massed in one part of the plant at the expense of any other part. Further confirmation of this condition is found in the data of BORST and THATCHER (**2**), which show that the absolute amount of nitrogen increases faster in the seeds than it decreases in the stems. GINSBURG and SHIVE (**12**) have also found that the

nitrogen content of normal soy beans was not appreciably altered by increasing the nitrogen concentration of the culture solution.

Data given in this report also indicate that nitrogen was not accumulated in exflorated plants, being but little higher than in the vegetative parts of control plants. On the other hand, the percentages of nitrogen were almost as high in the stems and leaves of exflorated and control plants as in flowering plants which were still vegetatively active. If lack of nitrogen assimilation had been the limiting factor in the growth of exflorated

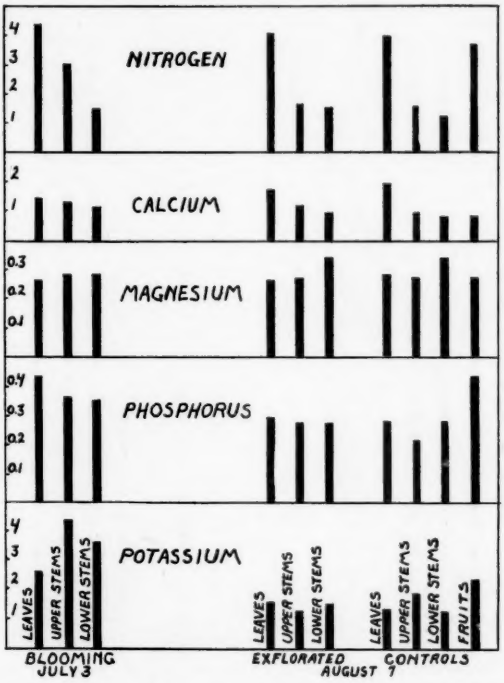


FIG. 3. Composition of soy beans; percentages in terms of dry weight.

plants, their nitrogen content should have been very low. Consequently, it may safely be concluded that the short day conditions which induce the reproductive phase do not entail nitrogen shortage, as suggested by NIGHTINGALE for *Salvia*; and further, there is no experimental evidence to substantiate the hitherto commonly accepted inference that the nitrogen demand of fruiting soy beans is so great as to deplete all reserves and thereby interfere with growth.

It is also to be noted that there was a slow rate of potassium intake after flowering as compared with the increase in dry weight and nitrogen, and this was coupled with decreased succulence of the tissues. These conditions are significant and probably important factors in the cessation of growth. NIGHTINGALE, SCHERMERHORN, and ROBBINS (29) found that potassium deficiency caused an early setting of fruit, lack of growth, accumulation of carbohydrates, and a low proportion of meristematic tissue in the tomato.

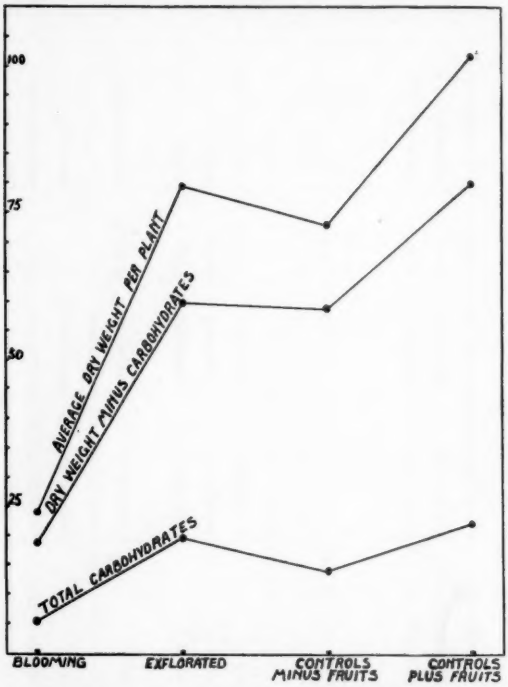


FIG. 4. Composition of soy beans; absolute weights in grams per plant.

They concluded that growth was limited because potassium is essential for synthesis of organic nitrogen from nitrates. In the case of the soy bean, however, a different interpretation is more plausible, namely, that potassium deficiency limited nitrogen assimilation only indirectly because the mechanism of cell division and growth was inhibited. The plant still had the ability to assimilate nitrogen if it could be utilized by some growing part of the plant. The controls assimilated nitrogen to supply the developing fruits, although potassium was at the same low level in the vegetative parts of the controls as in the exflorated plants. In the soy bean, therefore,

potassium must be essential for the later stages of protein synthesis, *i.e.*, probably for the condensation of amino acids. Hence potassium deficiency interferes with the formation of new protoplasm and limits meristematic activity in the stems.

As in the case of nitrogen, neither calcium, magnesium, potassium, nor phosphorus accumulated in the exflorated plants and yet the development of fruits did not deplete the reserves in the controls. This means that prac-

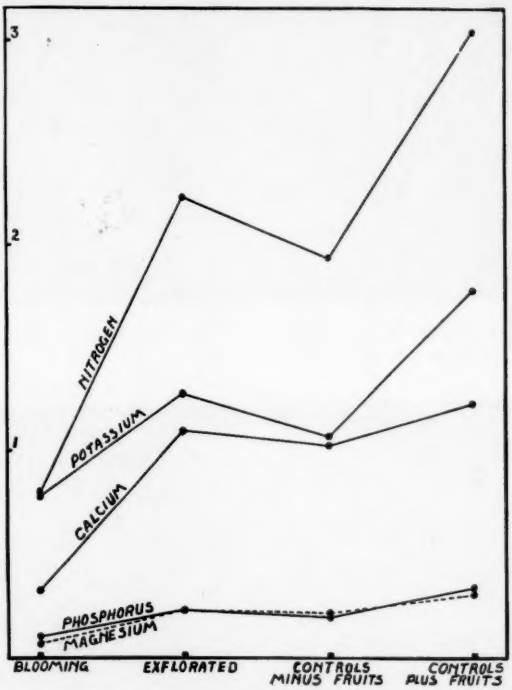


FIG. 5. Composition of soy beans; absolute weights in grams per plant.

tically the only substances stored in excess in the soy bean are carbohydrates; and that the plant, although inactive meristematically, still has the ability to take in nitrogen and minerals so that fruiting never becomes the exhaustive process which it is in some other plants (**23, 24**).

Arguments for and against the idea of balance between carbohydrates and nitrogen as the controlling factor in growth and reproduction of plants (**1, 6, 16, 18, 19, 28, 31, 32**) must consider the behavior of photoperiodic plants (**8, 9, 10, 11**). As already mentioned, *Salvia*, a short day plant, seems to be limited in its ability to assimilate nitrogen by a short day and

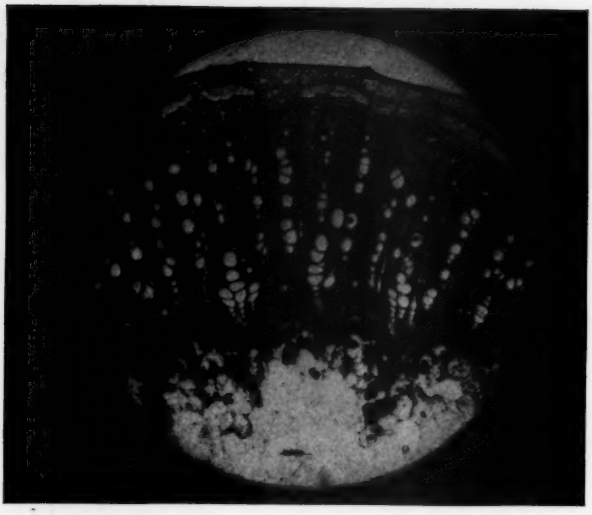

FIG. 6. Cross section of stem of exflorated soy bean plant stained with iodine to show storage of starch in medullary rays and pith.

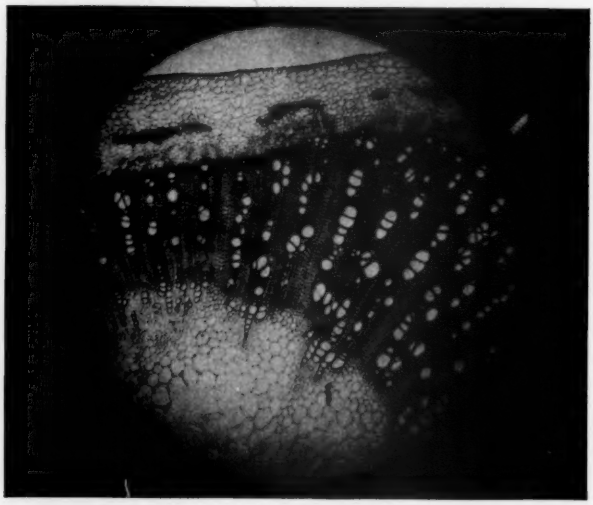

FIG. 7. Cross section of stem of normal control soy bean plant showing practically no starch (*cf.* fig. 6).

## TABLE III

ANALYSIS OF 1932 ITO SAN SOY BEANS; PERCENTAGES IN TERMS OF DRY WEIGHT

| DATE | PLANT PART | DRY WEIGHT | TOTAL NITROGEN | REDUCING SUGARS | TOTAL SUGARS | POLYSACCHARIDES | CALCIUM | MAGNESIUM | PHOSPHORUS | POTASSIUM |
|---|---|---|---|---|---|---|---|---|---|---|
| | | % | % | % | % | % | % | % | % | % |
| June 6 | Leaves | 14.45 | 5.20 | 2.67 | 4.16 | 11.34 | 2.12 | 0.377 | 0.440 | 2.29 |
| | Tip of stem | 9.04 | 4.20 | 6.30 | 8.70 | 12.49 | 1.32 | 0.296 | 0.611 | 5.08 |
| | Upper stem | 9.38 | 3.20 | 4.47 | 5.78 | 15.09 | 2.14 | 0.377 | 0.296 | 3.65 |
| | Lower stem | 15.26 | 2.02 | 2.23 | 2.67 | 16.62 | 1.58 | 0.285 | 0.212 | 1.89 |
| June 21 | Leaves | 17.77 | 4.56 | 3.27 | 4.13 | 12.89 | 2.02 | 0.356 | 0.378 | 1.76 |
| | Tip of stem | 8.58 | 3.76 | 7.91 | 8.57 | 16.54 | 1.05 | 0.235 | 0.621 | 5.70 |
| | Upper stem | 11.01 | 2.31 | 6.00 | 6.43 | 15.89 | 1.31 | 0.248 | 0.290 | 4.10 |
| | Lower stem | 17.16 | 1.67 | 3.50 | 3.89 | 17.51 | 1.07 | 0.249 | 0.242 | 2.58 |
| July 10; blooming | Leaves | 15.00 | 4.99 | 4.40 | 5.42 | 16.12 | 1.28 | 0.215 | 0.467 | 2.60 |
| | Tip of stem | 8.94 | 3.17 | 7.60 | 10.78 | 16.41 | 1.23 | 0.237 | 0.592 | 5.01 |
| | Upper stem | 10.92 | 2.01 | 6.88 | 8.80 | 21.07 | 1.27 | 0.260 | 0.304 | 4.35 |
| | Lower stem | 17.88 | 1.11 | 4.36 | 5.68 | 18.44 | 0.95 | 0.243 | 0.242 | 2.18 |
| August 18; exflorated | Leaves | 28.96 | 3.35 | 1.85 | 3.77 | 22.65 | 1.88 | 0.313 | 0.266 | 1.06 |
| | Tip of stem | 23.54 | 2.20 | 3.21 | 5.31 | 21.43 | 1.49 | 0.297 | 0.347 | 1.42 |
| | Upper stem | 27.29 | 1.78 | 2.12 | 4.03 | 24.53 | 0.96 | 0.323 | 0.279 | 0.89 |
| | Lower stem | 31.78 | 1.38 | 1.62 | 3.36 | 20.91 | 0.80 | 0.352 | 0.315 | 0.72 |
| August 18; controls | Leaves | 25.83 | 3.91 | 2.28 | 4.33 | 16.55 | 2.31 | 0.425 | 0.278 | 0.83 |
| | Tip of stem | 22.19 | 2.39 | 3.92 | 5.18 | 17.18 | 1.26 | 0.315 | 0.273 | 1.05 |
| | Upper stem | 23.56 | 1.68 | 4.03 | 5.39 | 18.27 | 1.04 | 0.394 | 0.310 | 0.81 |
| | Lower stem | 30.97 | 0.66 | 2.89 | 4.57 | 18.93 | 0.61 | 0.256 | 0.186 | 0.32 |
| | Fruits | 20.62 | 3.95 | 4.27 | 7.09 | 22.24 | 1.02 | 0.261 | 0.502 | 1.34 |

TABLE IV

SOY BEANS, AVERAGE TOTAL PERCENTAGES PER PLANT

| TIME SAMPLED | PERCENTAGES | | | |
|---|---|---|---|---|
| June 6, seedlings (dry wt. per plant 1.30 gm.) | Dry weight | 12.94 | Calcium | 2.01 |
| | Total sugars | 4.46 | Magnesium | 0.362 |
| | Polysaccharides | 12.30 | Phosphorus | 0.416 |
| | Total nitrogen | 4.62 | Potassium | 2.56 |
| June 21, vegetative (dry wt. per plant 7.61 gm.) | Dry weight | 15.79 | Calcium | 1.72 |
| | Total sugars | 4.56 | Magnesium | 0.318 |
| | Polysaccharides | 14.24 | Phosphorus | 0.356 |
| | Total nitrogen | 3.81 | Potassium | 2.36 |
| July 10, blooming (dry wt. per plant 21.57 gm.) | Dry weight | 14.18 | Calcium | 1.19 |
| | Total sugars | 6.34 | Magnesium | 0.231 |
| | Polysaccharides | 17.65 | Phosphorus | 0.384 |
| | Total nitrogen | 3.35 | Potassium | 2.92 |
| August 18, exflorated | Dry weight | 28.80 | Calcium | 1.47 |
| | Total sugars | 3.82 | Magnesium | 0.322 |
| | Polysaccharides | 22.54 | Phosphorus | 0.282 |
| | Total nitrogen | 2.61 | Potassium | 0.98 |
| August 18, controls (minus fruits) | Dry weight | 25.84 | Calcium | 1.65 |
| | Total sugars | 4.65 | Magnesium | 0.379 |
| | Polysaccharides | 17.40 | Phosphorus | 0.267 |
| | Total nitrogen | 2.73 | Potassium | 0.744 |
| August 18, controls (plus fruits) | Dry weight | 24.68 | Calcium | 1.54 |
| | Total sugars | 5.11 | Magnesium | 0.358 |
| | Polysaccharides | 18.30 | Phosphorus | 0.352 |
| | Total nitrogen | 2.96 | Potassium | 0.855 |

flowering accompanies the subsequent accumulation of carbohydrates. The experimental evidence for this conclusion, however, is very meager. In some long day plants the greatest accumulation of carbohydrates occurs under short day conditions, but flowering is initiated only by long days (**35, 36**).

From the results of ringing and defoliating experiments, HARVEY (**16**) claims that the vegetative responses of apple shoots are very definitely controlled by the ratio of carbohydrates to nitrogen. ROBERTS (**33**) and THOMAS (**34**) suggest that accumulation of carbohydrates interferes with nitrogen metabolism and retards growth. On the other hand, a high ratio of carbohydrates to nitrogen seems to be the result of inhibited or retarded growth and not the cause of it in many cases. HARTWELL (**15**) found that

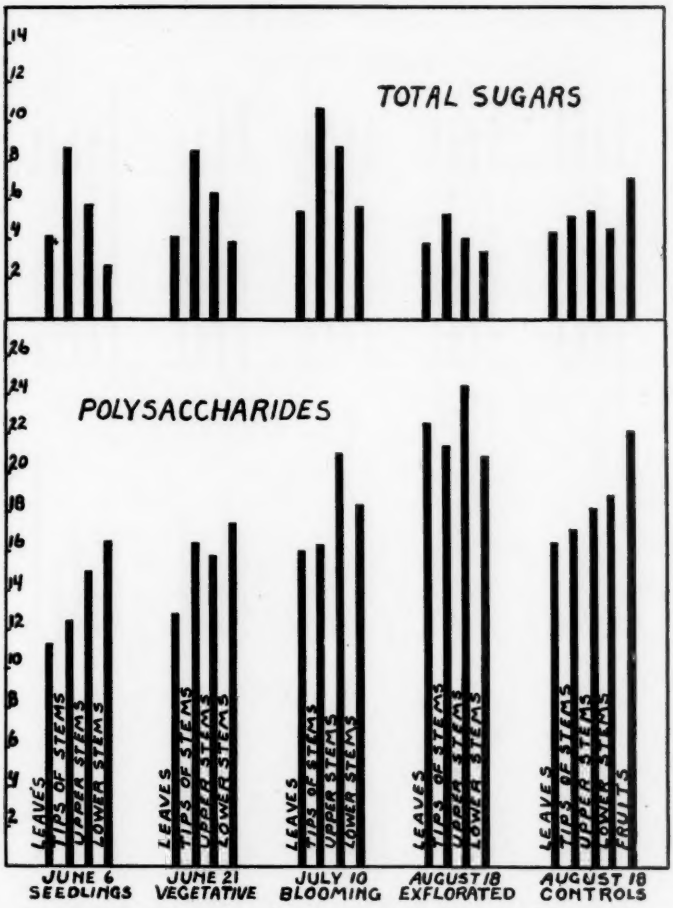


FIG. 8. Composition of soy beans; percentages in terms of dry weight.

accumulation of starch seemed to be correlated in general with conditions which caused a retardation of growth. If high carbohydrates limit growth, we still lack convincing experimental data to explain why long days stimulate growth in plants which have become high in carbohydrates under the influence of short days. This happens in some long day plants (**35, 36**), and GARNER and ALLARD (**8, 9**) have rejuvenated soy beans and other short day plants after they had begun to set fruits by subjecting them to long days.

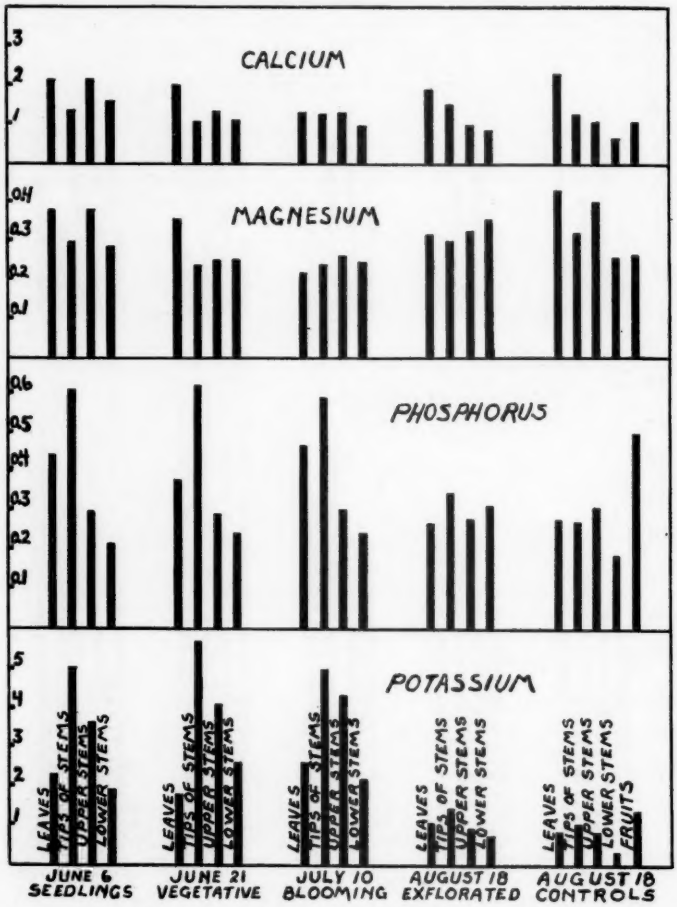


FIG. 9. Composition of soy beans; percentages in terms of dry weight.

The data for 1932 (tables III and IV and figs. 8–10) are given in full, although this involves some duplication, because they cover a full life cycle and because they represent a more detailed analysis of the plants than do those for 1931. These data disclose a high potassium content associated with high moisture content during the period of rapid growth, but nitrogen was not much higher in vegetative than in mature plants. Cessation of growth could hardly be attributed to a shortage of nitrogen, but the shortage of potassium probably had something to do with the decrease in mois-

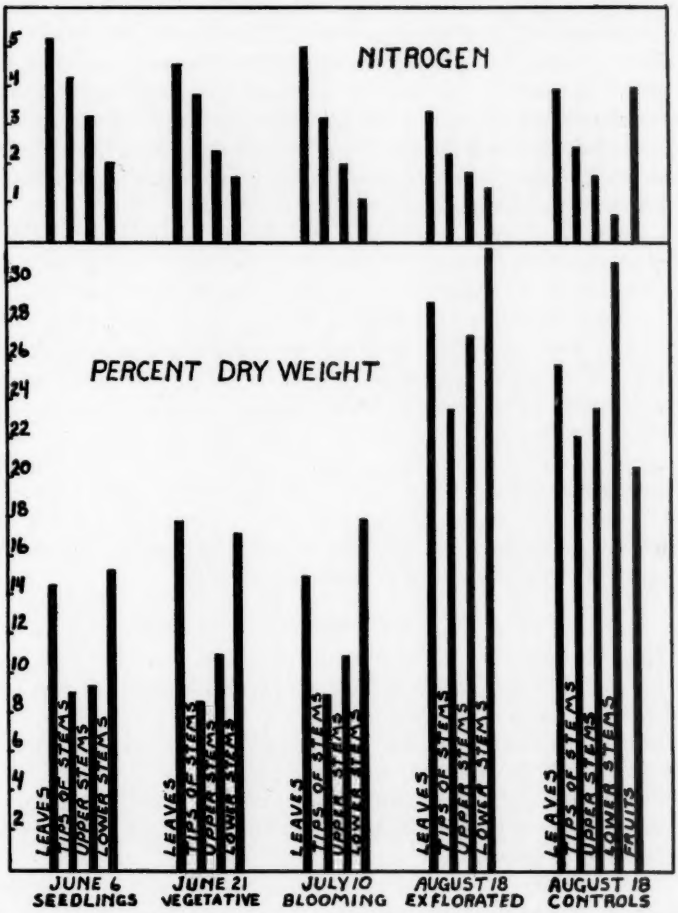


FIG. 10. Composition of soy beans; percentages of nitrogen in terms of dry weight and percentage dry weight in terms of fresh weight.

ture content and the retarded growth. Physiological dryness of tissues seems to be characteristic of plants deficient in potassium (**36, 38**). The appearance and behavior of exflorated soy bean plants are similar to those described by JANSSEN and BARTHOLOMEW (**17**) for tomato plants grown in a medium lacking in potassium. The percentages of phosphorus are highest in regions of rapid growth, stem tips of vegetative plants and fruits of control plants, but it does not decrease in other parts of the plant so much as does potassium when the plant stops growing.

Decreases in the percentages of potassium and moisture in the stems and leaves of mature soy bean plants are not due to fruiting since they are not prevented by exfloration. Likewise exfloration does not prevent cessation of growth. It seems likely then that the moisture, potassium, and to a less extent the phosphorus contents of the tissues are in some way related to nitrogen metabolism and the growth of new tissues. Whether these represent causes or only conditions accompanying particular growth responses cannot be stated with certainty.

In conclusion it may be stated that although growth of the soy bean plant normally stops at about the time that the fruits develop, plants from which the flowers are removed do not grow any larger than such normal plants. Hence cessation of growth is not due to fruiting, since it is not prevented by exfloration. Neither is it to be explained as a result of limited ability of the plant to absorb nitrogen. The best available explanation at present seems to be that the meristematic regions of the stem become inactive or dormant owing apparently to the decrease in day length, and the cessation of nitrogen absorption and the accumulation of carbohydrates are due to lack of growth. The exact mechanism of the action of the photoperiod is unknown, but in the soy bean it is related in some way to the moisture and potassium contents of the tissues. Exflorated soy bean plants show many of the symptoms of potassium starvation.

## Summary

1. In normal soy bean plants growth stopped at about the time that the fruits developed, which would suggest that fruit development was responsible for the cessation of growth.

2. Exfloration did not increase the vegetative development, growth ceasing simultaneously in exflorated and control plants.

3. The soy bean is a photoperiodic plant and apparently the shortening of the day length not only initiates the reproductive phase but also curtails the vegetative processes.

4. A shorter day is required to inhibit growth in the soy bean than is necessary to initiate flowering.

5. There was an abnormal accumulation of carbohydrates in exflorated plants.

6. Nitrogen did not accumulate in exflorated plants, but on the other hand it was not depleted in control plants by the development of fruits.

7. Neither calcium, magnesium, phosphorus, nor potassium was depleted in control plants by the development of fruits.

8. As the plants ceased to grow there was an increase in the percentage of dry weight, or in other words a decrease in moisture content, in both exflorated and control plants.

9. This decrease in moisture content and cessation of growth were associated with a marked decrease in the percentages of potassium in all parts of the stems and the leaves. Phosphorus decreased in the stem tips when the stems ceased to elongate.

10. The behavior of exflorated soy bean plants resembles the responses of some other plants to potassium starvation, and the suggestion is therefore made that the length of day may affect vegetative growth through its influence on the concentration of potassium in the tissues of the plant.

UNIVERSITY OF IOWA
IOWA CITY, IOWA

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# LIMITATIONS OF BLACKMAN'S LAW OF LIMITING FACTORS AND HARDER'S CONCEPT OF RELATIVE MINIMUM AS APPLIED TO PHOTOSYNTHESIS

B. N. SINGH AND K. N. LAL

(WITH NINE FIGURES)

## Problem and mode of attack

The universality of the photosynthetic process and its fundamental nature and importance have long been recognized, and little is known regarding the systems involved and their exact working in consonance with both external and internal factors. From the time it came to be recognized that $CO_2$ was absorbed by the leaves during photosynthesis and that with an increase in its concentration the rate of assimilation increased, attempts have been made to determine an optimum concentration of carbon dioxide, a percentage which may induce rapid photosynthesis, leading to increased growth in plants.

With the formulation of LIEBIG's law of the minimum (**7**) and with the advance in our knowledge of the subject of photosynthesis, it came to be realized that the process is conditioned by numerous factors. PFEFFER (**12**) and PANTANELLI (**13**) realized that the optimum value in photosynthesis was not fixed under the influence of any single factor.

Considerable work has been carried out in recent years on the relationship of photosynthesis to external factors, and several laws indicating the possible relationship between the external factors on the one hand and carbon assimilation on the other have been evolved.

BLACKMAN (**1**) first called attention to the fact that in a phenomenon such as photosynthesis, the focusing of attention on a single factor in disregard to the influence of others leads to erroneous results. A study of the interrelation of the conditioning factors led him to formulate the principle of limiting factors, which he has stated in the following axiom: "When a process is conditioned as to its rapidity by a number of separate factors the rate of the process is limited by the pace of the slowest factor." This theory stimulated investigations designed to test the validity of BLACKMAN'S proposals.

His observations were confirmed by MATTHAEI (**11**), BLACKMAN and SMITH (**2**), and WILMOTT (**16**), but later workers like HOOKER (**5**), BROWN (**4**), BOYSEN JENSEN (**3**), and HARDER (**6**) criticized the theory and showed that the law of limiting factors was not infallible. This theory was subsequently modified by HARDER (**6**) in his concept of relative minimum, but at present both theories seem to be equally supported on an experimental

basis. One of the vital points of difference between the viewpoints of BLACKMAN and HARDER is that while the former holds that there is a sudden change in direction of the ascending curve to a horizontal one when the limiting factor comes into operation, the latter holds that the curves are extremely regular and that there is no sudden change of the ascending curve to a horizontal phase. Moreover, the rate is not governed by one factor and that alone, as was supposed by BLACKMAN, but is also conditioned by the intensity of others present in relative minimum. Both these observations are equally supported by experimental data and the question therefore remains, Is there a sharp break in the curve or is it smooth in form?

The present work is the outcome of a study concerned with the nature of growth in relation to external and internal factors. In trying to study growth with respect to other metabolic activities, more particularly assimilation and respiration, it was necessary to determine under laboratory conditions the relation between $CO_2$ assimilation and such external factors as temperature, light, and carbon dioxide. Special effort has been made to estimate their effect on the rate of photosynthesis when they are present in concentrations and intensities in which they naturally occur in the field.

The results have thrown considerable light on the nature as well as on the applicability of the law of limiting factors and the theory of relative minimum, and these have therefore received separate treatment in the present paper.

## Investigation

MATERIAL.—Material was selected from crops of economic importance such as wheat (var. Pusa 4), linseed (var. 1150 S), and sugar cane (var. Reori) grown both under natural environment and under conditions of optimum nutritional supply at the Experimental Farm of this Research Station.

APPARATUS.—The apparatus consists of a carbon dioxide absorbing set, a $CO_2$ generating tower, a constant temperature bath, and a commutator and clockwork arrangement identical in most respects with the one designed by BLACKMAN for investigations on vegetable assimilation. The plant chamber is of special construction, with inlet and exit tubes, an opening at the top for the insertion of a thermometer, and a depression for the attachment of a small funnel containing water into which the leaf base is dipped. The front and back sides of the chamber are of glass, one pane of which can easily be removed for admitting the experimental leaf.

Half-watt 220-volt Phillips bulbs of various intensities were used as the source of illumination. The exact intensity in each case was determined by comparison with standard bulbs and the values expressed in candle

power. A screen of running water intervened between the bulb and the plant chamber in order to prevent heat from the bulb reaching the bath.

The temperature of the bath was maintained constant for each series of experiments by means of thermo-gas regulators. The experimental temperature varied between 23° and 40° C., and never fluctuated more than $\pm$ 0.05° C.

The $CO_2$ concentration used was between 0.0 and 0.5 per cent., according to the requirement of the experiment. The percentage of carbon dioxide by volume was calculated by the formula:

$$\text{percentage } CO_2 = \frac{\text{wt. of gas in control tubes per hr. in gm.} \times 500}{1500} \times 100,$$

where 500 is the approximate volume of the gas weighing 1 gm. and 1500 the speed of dropping in the aspirators per hour.

The details of temperature, light intensity, and $CO_2$ concentration in a particular experiment are given in the tables.

## Experimental procedure

The general procedure consisted in the selection of mature healthy normal green leaves from an average plant of known age a day previous to the time of the experiment. These leaves were kept near a north window, covered with an open belljar with their ends in water and left over night to attain laboratory conditions. The leaves were regularly collected at 4 P. M. after a period of rapid assimilation and the experiment was started each day at about 10 A. M. after recording the fresh weight and the area of the assimilating leaf surface.

The leaves prepared in this manner were arranged in the assimilation chamber and connections were then made to the $CO_2$ generating set and the commutator. A current of air containing a definite amount of carbon dioxide was divided into two equal parts. One was passed over the experimental leaf and was then withdrawn through a Pettenkoffer tube containing a measured quantity of standard baryta solution. The other part was passed directly through another Pettenkoffer tube containing the same amount of barium hydroxide. After a definite interval, the two tubes were disconnected, their contents poured into separate beakers, and the solution titrated against standard hydrochloric acid. The difference in the acid value of the two solutions is due to the reduction in the amount of $CO_2$ absorbed by the assimilating leaf. A preliminary tube was always run for 1.5 hours before taking the actual readings, followed by three consecutive hourly readings for the determination of the apparent assimilation. The respiration of the same material under identical conditions, but in complete darkness, was determined and the real assimilation calculated for 100 sq. cm. of assimilating area per hour. For each experiment a new leaf was used.

Care was taken to determine experimentally the amount of external carbon dioxide absorbed by the solution during the course of filling, fixing, and titrating the solution. This "washing factor" was determined several times during the course of the experiment and due allowance made for it in the readings.

Precaution was also taken to avoid as far as possible the resistances in the diffusion and photochemical phases of the process (**8, 9**), or to bring them to a common factor throughout the whole series. Variations due to season were avoided by performing the experiment in a single season of the year. The observations were taken at definite hours of the day and under definite artificial illumination, thus reducing variation in resistance due to time of day, intensity of illumination, and time of beginning of the experiment. To avoid the effect of previous history of the leaf only healthy leaves from average selected plants were taken and kept with the leaf base in water for several hours to equalize moisture conditions. Only leaves of the same developmental stage were used.

The course of assimilation at various concentrations and combinations of external factors was followed with only the mature leaves during the adolescent phase of the life cycle.

## Results

The data are mainly confined to three of the most important external factors, light, temperature, and carbon dioxide, which have been shown by previous workers to have considerable influence on the rate of photosynthesis.

### $CO_2$ concentration and assimilation

With the preliminary precautions already described, observations were made on the effect of carbon dioxide on the rate of assimilation. Concentrations both below and above that present in the field were used, the former being maintained by absorbing some of the $CO_2$ in the current by potash and the latter by providing extra quantities of this gas in the stream by the $CO_2$ generating tower.

Since the experiments were conducted entirely under laboratory conditions, other factors like light intensity and temperature were also controlled. Under a constant temperature and a constant light intensity, the $CO_2$ assimilation was determined at 0.02 per cent. external concentration of carbon dioxide. The results are set forth in table I, experiment 1.

Under the conditions of the experiment, it is evident that the green leaf is not in a position to obtain any appreciable quantity of carbon dioxide from its surroundings. It has naturally to depend on the $CO_2$ evolved during respiration for the synthesis of carbohydrates. The quantity of

TABLE I

EFFECT OF $CO_2$ CONCENTRATION ON RATE OF CARBON ASSIMILATION BY MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.

ILLUMINATION, 90 C.P. HALF-WATT PHILLIPS BULB AT 16 CM. DISTANCE

| DATE (1932) | EXPERIMENT NO. | $CO_2$ CONCENTRATION | REAL ASSIMILATION PER 100 SQ. CM. PER HR. |
|---|---|---|---|
| | | % | *mg.* |
| July 1 | 1 | 0.02 | 0.403 |
| " 2 | 2 | 0.051 | 1.050 |
| " 3 | 3 | 0.0806 | 1.150 |
| " 4 | 4 | 0.1280 | 1.005 |
| " 5 | 5 | 0.3740 | −0.960 |

respiratory carbon dioxide for the entire leaf area being small, the values of assimilation are naturally low.

The carbon assimilation under slightly greater concentration of carbon dioxide, *viz.*, equivalent to that of the atmosphere, was then determined. The results are shown in table I, experiment 2. In this case assimilation is higher than the preceding, indicating that with increase in the percentage of carbon dioxide the value of assimilation increased.

To test whether an increase in assimilation will follow a further increase in the percentage of carbon dioxide, the concentration was raised to 0.0806 per cent. Experiment 3 indicates that the intensity of assimilation does increase.

Experiments 4 and 5 were designed to study the effect of still higher concentrations of carbon dioxide on the rate of photosynthesis. Light intensity and temperature being kept the same as before, the variations found in the readings should be due to the increase in the concentration of carbon dioxide.

The rates of assimilation obtained under different concentrations of carbon dioxide (table I) show that up to 0.08 per cent. of carbon dioxide the velocity of the reaction increases, and any further increase is accompanied by a decrease in the intensity which finally attains a negative value under 0.374 per cent. It would thus follow that after 0.08 per cent. carbon dioxide concentration any extra quantity of carbon dioxide produces some sort of inhibitory effect on the process, the intensity of inhibition increasing with an increase in its concentration.

It has just been shown that assimilation under the particular temperature and light intensity is highest at 0.08 per cent., the readings falling

gradually on both sides of this maximum. Why should the intensity of photosynthesis rise for some time and then decline with increasing concentration of carbon dioxide? In the light of BLACKMAN'S concept we can explain it as due to the fact that the carbon dioxide supply up to this stage is limiting; hence an increase in the limiting factor, the pace of the slowest factor, increases the rate of assimilation. Following HARDER, the same phenomenon may be explained on the basis that the carbon dioxide is at first lesser in quantity or in relative minimum than other conditioning factors which are exerting greater influence over the process. And as its concentration is increased the intensity becomes still greater, finally equaling that of other factors in which the readings are maximum. The subsequent fall with increase in $CO_2$ concentration may be due to the deleterious effect of excessive carbon dioxide.

Whatever explanation we may give to the nature of the phenomenon, it is clear from the data obtained (fig. 1, 90 c.p.) that with increasing concentration of $CO_2$ the intensity of photosynthesis increases in regular sequence. The sudden transference of the ascending phase into a horizontal one, as was observed by BLACKMAN and his co-workers, is not apparent in the present case. Instead the curve shows a gradual rounding, thus probably answering one of the vital questions raised in our introduction. Further, the stationary phase when the limiting factor comes into operation as observed by BLACKMAN is not marked in the present case. If it is present at all, it may be found only within a small range of concentration. The decline phase on the other hand is characteristically evident.

In order to test the validity of these results obtained for sugar cane, inquiry along strictly similar lines was conducted on wheat and flax plants grown side by side under similar conditions. The gradations of the carbon

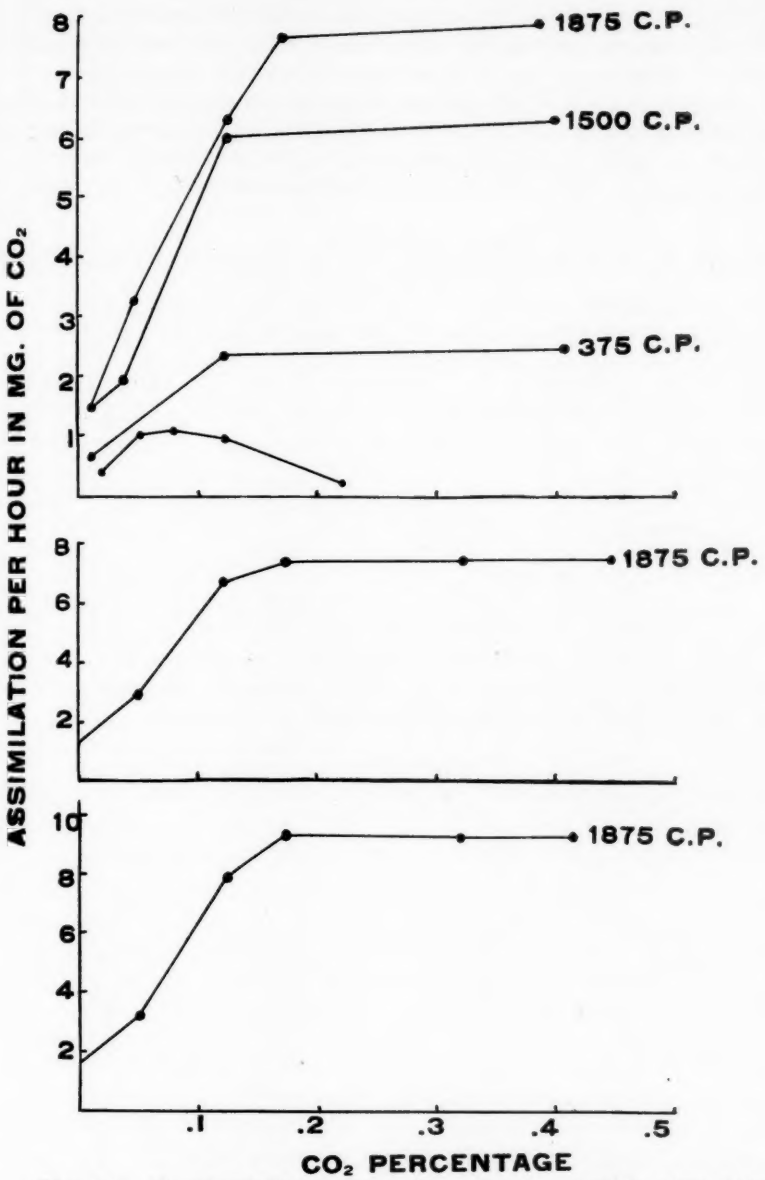
TABLE II

EFFECT OF $CO_2$ CONCENTRATION ON RATE OF CARBON ASSIMILATION BY MATURE LEAVES OF WHEAT, PUSA 4

TEMPERATURE, 30.0° C.

ILLUMINATION 1875 C.P. HALF-WATT PHILLIPS BULB AT 16 CM. DISTANCE

| DATE (1932) | EXPERIMENT NO. | $CO_2$ CONCENTRATION | REAL ASSIMILATION PER 100 SQ. CM. PER HR. |
|---|---|---|---|
| | | % | *mg.* |
| March 11 | 22 | 0.05 | 2.82 |
| " 11 | 29 | 0.124 | 6.75 |
| " 12 | 30 | 0.172 | 7.37 |
| " 13 | 31 | 0.320 | 7.40 |




FIGS. 1–3. $CO_2$ and assimilation curves. Above, sugar cane; middle, wheat; below, flax. Illumination in candle power. Lowest curve for sugar cane, 90 c.p.

dioxide range as well as the constancy of temperature and light intensity were maintained, although the magnitude of each was purposely raised in order to compare our data at higher intensities of the factors.

In spite of material difference in the leaves which were gathered from plants of three different types, curves (figs. 2, 3) differing only in magnitude but essentially similar in nature to the one obtained for sugar cane were secured in these two cases as well (tables II and III).

TABLE III

EFFECT OF $CO_2$ CONCENTRATION ON RATE OF CARBON ASSIMILATION BY MATURE LEAVES OF LINSEED, 1150 S

TEMPERATURE, 30.0° C.
ILLUMINATION, 1875 C.P. HALF-WATT PHILLIPS BULB AT 16 CM. DISTANCE

| DATE | EXPERIMENT NO. | $CO_2$ CONCENTRATION | REAL ASSIMILATION PER 100 SQ. CM. PER HR. |
|---|---|---|---|
| | | % | *mg.* |
| March 8, 1932 | 25 | 0.05 | 3.24 |
| February 1, 1933 | 26 | 0.124 | 7.94 |
| " 2, 1933 | 27 | 0.172 | 9.23 |
| February 3, 1933 | 28 | 0.32 | 9.23 |

Medium and high intensities of light were also used on sugar cane to test the nature of the reaction as affected by increased illumination. Table IV includes three experiments with medium light intensity.

TABLE IV

EFFECT OF $CO_2$ CONCENTRATION ON RATE OF CARBON ASSIMILATION BY MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.
ILLUMINATION, 375 C.P. HALF-WATT PHILLIPS BULB AT 16 CM. DISTANCE

| DATE (1932) | EXPERIMENT NO. | $CO_2$ CONCENTRATION | REAL ASSIMILATION PER 100 SQ. CM. PER HR. |
|---|---|---|---|
| | | % | *mg.* |
| May 7 | 6 | 0.011 | 0.680 |
| " 8 | 7 | 0.121 | 2.391 |
| " 9 | 8 | 0.407 | 2.510 |

The readings (table IV) show an initial rapid increase in the rate of assimilation, followed by a more or less stationary phase. In this case

there is a wider range in assimilation than in the previous case. The intensity of the reaction is greater in the present case, however, because of the greater supply of energy now available.

A series of experiments was performed also with higher light intensity. In this series, as is evident from the curves shown in figure 1 and the values in tables V and VI, the nature of the reaction as judged by the rate of assimilation remains the same, differing only in intensity and magnitude.

TABLE V

EFFECT OF $CO_2$ CONCENTRATION ON RATE OF CARBON ASSIMILATION BY MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.

ILLUMINATION, 1500 C.P., HALF-WATT PHILLIPS BULBS AT 16 CM. DISTANCE

| DATE (1932) | EXPERIMENT NO. | $CO_2$ CONCENTRATION | REAL ASSIMILATION PER 100 SQ. CM. PER HR. |
|---|---|---|---|
| | | % | *mg.* |
| July 12 | 10 | 0.011 | 1.48 |
| " 11 | 9 | 0.036 | 1.964 |
| " 13 | 11 | 0.124 | 6.100 |
| " 18 | 12 | 0.403 | 6.38 |

TABLE VI

EFFECT OF $CO_2$ CONCENTRATION ON RATE OF CARBON ASSIMILATION BY MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.

ILLUMINATION, 1875 C.P., HALF-WATT PHILLIPS BULBS AT 16 CM. DISTANCE

| DATE (1932) | EXPERIMENT NO. | $CO_2$ CONCENTRATION | REAL ASSIMILATION PER 100 SQ. CM. PER HR. |
|---|---|---|---|
| | | % | *mg.* |
| July 19 | 13 | 0.0476 | 3.303 |
| " 20 | 14 | 0.124 | 6.290 |
| " 21 | 15 | 0.172 | 7.770 |
| " 22 | 16 | 0.388 | 7.920 |

It is thus seen (table VI) that a higher light intensity increases the rate of photosynthesis several-fold. The higher rate is possibly correlated with the greater supply of available energy, and in fact it was not practicable within the limited time at our disposal actually to determine the concentra-

tions at which the toxic or inhibitory effect comes into prominence, since the leaves could endure sufficiently high $CO_2$ concentration, up to ten times higher than that in the field, under very high intensities. Several readings were not possible for each light intensity since the investigations necessarily had to be finished within a limited stage in the life cycle of crops in order to avoid the effect of age and developmental stage on assimilation.

The curves of figure 1 indicate that with the higher intensities of light, the ascending phase becomes more and more characteristic and prominent. The magnitude of the ascending phase in contradistinction to the level phase is greater as we use higher light intensities, and it may therefore be expected that if an unlimited supply of light energy be available, together with a continuous and increasing supply of carbon dioxide, the inflexion phase may be entirely lost, the reaction proceeding in a linear fashion. But how far such a type of curve can be expected in practice is a matter for experimentation. It is just possible that, even with all the external factors supplied at maximum level, the nature of the reaction may remain the same on account of the control of the process by physiological or internal factors, namely, the specificity of the plant, the nature of the products formed, the chlorophyll apparatus, the water content, etc.

### Light intensity and assimilation

That light is essential for photosynthesis is well known, but owing to the complexities introduced by the simultaneous influence of other factors, carbon dioxide and temperature, it becomes difficult to gauge the relationship at its correct value. For example, as already shown, the influence of carbon dioxide on the rate of assimilation is found to be dependent upon light, the rate rising with increased intensity. It is also known that temperature influences the rate, a rise being obtained by raising the temperature until a certain degree is reached followed by a decline with further rise in temperature. These facts lead one to conclude that in investigating the influence of light, the simultaneous influence of two other factors, carbon dioxide and temperature, must be taken into consideration.

In the present study, which does not aim at a detailed investigation of the problem but is simply concerned with the determination of the light intensity required to deplete the normal concentration of carbon dioxide, the effect of increasing light intensity on the rate of photosynthesis has been tested at four different concentrations of $CO_2$ both below and above that normally present in air.

Zero percentage external $CO_2$ concentration.—Under such a condition the plant is not in a position to utilize any other $CO_2$ except that which it is itself evolving during the downgrade process of respiration. The readings obtained under such conditions are given in table VII.

## TABLE VII

EFFECT OF LIGHT INTENSITY ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.

$CO_2$ CONCENTRATION, 0.0 PER CENT.

| DATE (1932) | EXPERIMENT NO. | ILLUMINATION | REAL ASSIMILATION PER 100 SQ. CM. PER HR. |
|---|---|---|---|
| | | *c.p.* | *mg.* |
| July 1 | 1 | 90 | 0.403 |
| " 7 | 6 | 375 | 0.680 |
| " 12 | 10 | 1500 | 1.480 |
| " 13 | 40 | 1875 | 1.480 |

It is seen that the rates of assimilation at 90, 375, 1500, and 1875 c.p. are 0.4, 0.68, 1.48, and 1.48 mg. respectively per hour. This shows that the respiratory carbon dioxide, which is the only carbon dioxide available for photosynthesis under such circumstances, is not all used at lower light intensities. Experiments 1 and 6, dealing with 90 and 375 c.p. respectively, show that under these light intensities the plant is not in a position to synthesize what it respires. As the light intensity is increased the photosynthetic value rises, and ultimately the leaf is able to utilize the total output of carbon dioxide (experiment 10). When the two processes balance each other, *i.e.*, when the compensation point is reached, no more increase in the readings is obtained with further increase in light intensities.

What is the nature of the reaction at this stage, namely, how far does the photosynthetic chain of reactions proceed and how are they correlated with the opposite series of reactions connected with the respiratory process? Answers to these questions may throw light on the nature of the two interrelated reactions.

ATMOSPHERIC CONCENTRATIONS OF $CO_2$.—Since all the plants grew under this concentration it was thought advisable to investigate the effect of increasing illumination on the rate of photosynthesis under such atmospheric percentage of $CO_2$ in the case of different plants. Besides the sugar cane leaves which were subjected to detailed investigation, leaves of wheat and flax were experimented upon also.

The readings obtained for sugar cane leaves under this concentration and different light intensities are shown in table VIII.

It is evident that with higher light intensities (90 to 1875 c.p.) the rate of assimilation increases from 1.05 to 2.23 mg. Even at the high light intensity of 1500 c.p. the readings show an increase when the intensity is

## TABLE VIII

EFFECT OF LIGHT INTENSITY ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.
CONCENTRATION, 0.05 PER CENT.

| DATE (1932) | EXPERIMENT NO. | ILLUMINATION | REAL ASSIMILATION PER 100 SQ. CM. PER HR. |
|---|---|---|---|
| | | *c.p.* | *mg.* |
| July 2, 1932 | 2 | 90 | 1.05 |
| March 6, 1933 | 17 | 375 | 1.52 |
| " 7, 1933 | 18 | 1500 | 2.1 |
| " 8, 1933 | 19 | 1875 | 2.23 |

increased to 1875 c.p. The constant phase of the activity could not be determined on account of lack of higher light intensities. The rates of assimilation of wheat leaves under the different light intensities and atmospheric concentration of $CO_2$ are shown in table IX.

## TABLE IX

EFFECT OF LIGHT INTENSITY ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF WHEAT, PUSA 4

TEMPERATURE, 30.0° C.
$CO_2$ CONCENTRATION, 0.05 PER CENT.

| DATE (1933) | EXPERIMENT NO. | ILLUMINATION | REAL ASSIMILATION PER 100 SQ. CM. PER HR. |
|---|---|---|---|
| | | *c.p.* | *mg.* |
| March 9 | 20 | 375 | 2.05 |
| " 10 | 21 | 1500 | 2.74 |
| " 11 | 22 | 1875 | 2.82 |

On comparing the rates (table IX), it is clear that the results with wheat are rather similar, the rate rising with the light intensity. The readings at 1500 and 1875 c.p. are 2.74 and 2.82 mg. There is not much difference between the two, however, and we can say that 1875 c.p. is sufficient to deplete all the carbon dioxide normally present in the atmosphere.

Linseed plants showed contrasting results. The readings at 375, 1500, and 1875 c.p. are shown in table X. The rate of assimilation does not rise with increase in light intensity beyond 1500 c.p.

## TABLE X

EFFECT OF LIGHT INTENSITY ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF LINSEED, 1150 S

TEMPERATURE, 30.0° C.

$CO_2$ CONCENTRATION, 0.05 PER CENT.

| DATE (1932) | EXPERIMENT NO. | ILLUMINATION | REAL ASSIMILATION PER 100 SQ. CM. PER HR. |
|---|---|---|---|
| | | *c.p.* | *mg.* |
| March 16 | 23 | 375 | 2.72 |
| " 17 | 24 | 1500 | 3.24 |
| " 18 | 25 | 1875 | 3.24 |

From the results obtained with regard to the influence of light on the photosynthetic rate of the three crop plants, it is seen that while light intensity of 1875 c.p. and more is necessary for maximal values in the cases of wheat and sugar cane, an intensity of 1500 is sufficient for linseed. In other words, linseed plants under normal conditions of $CO_2$ require less light intensity for attaining their optimum value than do sugar cane and wheat. Whether such differences are in any way connected with the nature of the end product of the upgrade process (sugar in sugar cane, starch in wheat, and oil in linseed) is a problem requiring further investigation.

MEDIUM $CO_2$ CONCENTRATION.—Only the sugar cane leaves were the subject of experimentation under medium concentration of carbon dioxide. The rates of assimilation are shown in table XI.

## TABLE XI

EFFECT OF LIGHT INTENSITY ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.

$CO_2$ CONCENTRATION, 0.12 PER CENT.

| DATE (1932) | EXPERIMENT NO. | ILLUMINATION | REAL ASSIMILATION PER 100 SQ. CM. PER HR. |
|---|---|---|---|
| | | *c.p.* | *mg.* |
| July 4 | 4 | 90 | 1.005 |
| " 8 | 7 | 375 | 2.391 |
| " 13 | 11 | 1500 | 6.10 |
| " 20 | 14 | 1875 | 6.29 |

There is a clear and characteristic rise similar to that observed under normal concentrations above, but differing in magnitude. Under such a concentration the rise is more or less proportional to light intensity (fig. 4). The stationary phase could not be secured, owing to the lack of sufficiently strong light which we had not at our disposal when the investigations were in progress.

OPTIMUM $CO_2$ CONCENTRATION.—In this case the values as shown in table XII indicate the rate of photosynthesis of sugar cane leaves.

TABLE XII

EFFECT OF LIGHT INTENSITY ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF SUGAR CANE, VAR. REORI

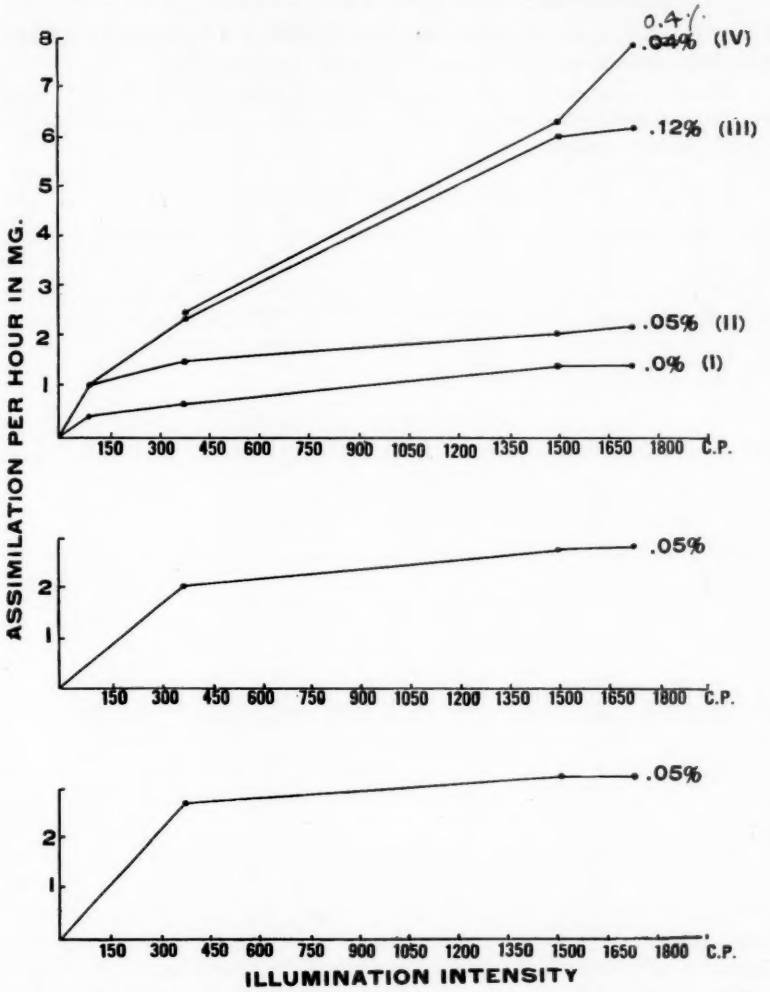
TEMPERATURE, 30.1° C.

$CO_2$ CONCENTRATION, 0.4 PER CENT.

| DATE (1932) | EXPERIMENT NO. | ILLUMINATION | REAL ASSIMILATION PER 100 SQ. CM. PER HR. |
|---|---|---|---|
| | | *c.p.* | *mg.* |
| July 5 | 5 | 90 | −0.96 |
| " 9 | 8 | 375 | 2.51 |
| " 18 | 12 | 1500 | 6.38 |
| " 22 | 16 | 1875 | 7.92 |

The curves shown in figure 4 display a direct proportionality between light intensity and the rate of assimilation from 375 c.p. onward, showing that light governs the reaction under conditions of optimum $CO_2$ concentration. At 90 c.p. the intensity of light is too feeble for this high concentration of $CO_2$, under which circumstances the carbon dioxide exerts its toxic effect as shown by negative photosynthesis.

The nature of the reaction as measured by the $CO_2$ intake under different concentrations of $CO_2$ at different light intensities (fig. 4) shows at first an ascending phase but the steepness of the curve depends upon the concentration of carbon dioxide. The richer the environment the higher is the angle of curvature and the higher the values. In the case of a zero percentage $CO_2$ concentration the curves become flattened when light intensity is raised from 1500 to 1875 c.p., showing that under these conditions $CO_2$ is limiting the rate. But such a stationary phase was not observed at 1500 and 1875 c.p. in the case of higher concentrations. This shows that under higher concentrations of carbon dioxide the existence of the stationary phase requires very high light intensity, a theory which has still to be experimentally supported.




FIGS. 4–6. Light and assimilation curves. Above, sugar cane; middle, wheat; below, flax.

In the light of BLACKMAN's theory, curve no. 1 (fig. 4) can be explained as consisting of two parts, an ascending phase when light is limiting and a stationary one when $CO_2$ is limiting. In the case of other curves we have only the ascending phase, indicating a stage when light is always limiting. The curves can equally be explained on the basis that during the ascending

phase the light intensity is in relative minimum and during the stationary phase the other factor, $CO_2$, falls short of the requirement.

We shall discuss critically the relative importance of the theory of limiting factors and that of relative minimum after we have dealt with the third factor, namely, temperature.

### Temperature and assimilation

Under ordinary conditions in the field, the $CO_2$ concentration during the day does not increase to a great extent. The values are more or less stationary, but so far as the temperature is concerned it shows decidedly wide fluctuations. In order to determine the optimum temperature under normal concentration of carbon dioxide, a series of experiments as shown in table XIII was performed.

TABLE XIII

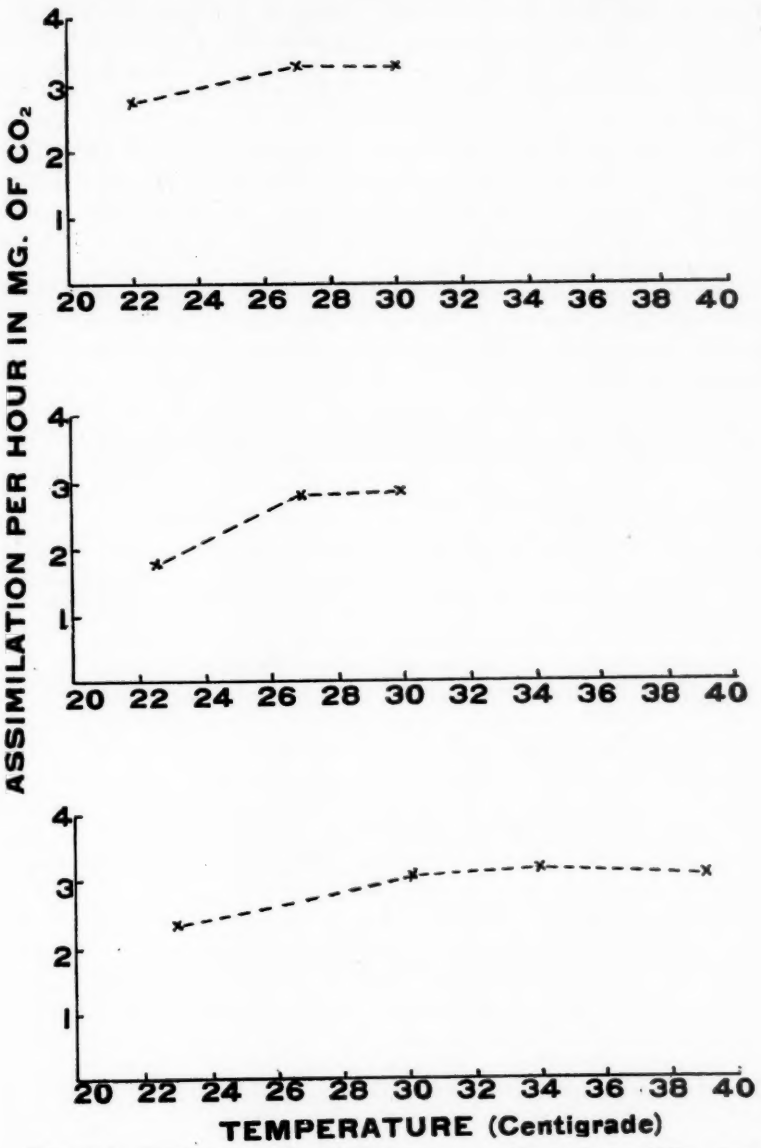
Effect of temperature on rate of carbon assimilation of mature leaves of sugar cane, var. Reori

$CO_2$ concentration, 0.05 per cent.
Light intensity, 1875 c.p.

| Date (1932) | Experiment no. | Temperature | Real assimilation per 100 sq. cm. per hr. |
|---|---|---|---|
| | | °C. | mg. |
| July 19, 1932 | 13 | 30.1 | 3.03 |
| March 9, 1933 | 36 | 23.0 | 2.3 |
| " 11, " | 38 | 34.0 | 3.2 |
| " 12, " | 39 | 39.0 | 3.05 |

Only four temperatures, which came within the purview of fluctuations within these regions and to which the plants are subjected at this time of the year, were selected: 23°, 30°, 34°, and 39° C., it being considered unnecessary to go above or below these temperatures. In each case light intensity sufficiently high so as not to limit the rate was used. The concentration of carbon dioxide was the same as that obtained in the field.

From the values shown (figs. 7–9), it is clear that the rate of assimilation rises with rise in temperature for some time, followed by a decline. Two influences seem to be at work: first, the effect of temperature in raising the amount of assimilation of which the leaf is capable; second, an injurious effect which becomes more and more prominent with high temperature. The rate at any point is therefore the result of an accelerating as well as of a depressing effect of temperature. But at higher temperatures a




FIGS. 7–9. Temperature and assimilation curves. Above, flax; middle, wheat; below, sugar cane.

point is reached when the acceleration due to increased temperature is balanced by the depressing action. This point gives the maximum assimilation and lies for sugar cane leaves near 34° C.; but, as may be seen from the individual readings in experiment 38, the initial value is not maintained very long.

At temperatures higher than 34° C. the injurious effect gains ascendency and there is a fall in the rate of photosynthesis. The initial values are not maintained as can be seen from the individual readings in experiment 39. There is a rapid fall from hour to hour. The time effect sets in very soon at higher temperatures.

A temperature near the optimum most suitable for study of photosynthesis would be about 30° C. At such a temperature the time effect is not marked for a sufficient period, as shown by the constancy of the readings in experiment 37.

TABLE XIV

EFFECT OF TEMPERATURE ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF WHEAT, PUSA 4

$CO_2$ CONCENTRATION, 0.05 PER CENT.
LIGHT INTENSITY, 1875 C.P.

| DATE (1933) | EXPERIMENT NO. | TEMPERATURE | REAL ASSIMILATION PER 100 SQ. CM. PER HR. |
|---|---|---|---|
| March 14 | 32 | 22.5 | 1.72 |
| " 15 | 33 | 27.0 | 2.8 |
| " 11 | 22 | 30.0 | 2.82 |
| | | °C. | mg. |

TABLE XV

EFFECT OF TEMPERATURE ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF LINSEED, 1150 S

$CO_2$ CONCENTRATION, 0.05 PER CENT.
LIGHT INTENSITY, 1875 C.P.

| DATE (1932) | EXPERIMENT NO. | TEMPERATURE | REAL ASSIMILATION PER 100 SQ. CM. PER HR. |
|---|---|---|---|
| | | °C. | mg. |
| February 9 | 34 | 22.0 | 2.72 |
| " 10 | 35 | 27.0 | 3.22 |
| March 18 | 25 | 30.0 | 3.24 |

With regard to the nature of the reaction, the studies show that VAN'T HOFF'S temperature coefficient between 23° and 33° C. would be in the vicinity of 1.4, characteristic of photochemical reactions. As to the exact nature of this reaction and the part it has in the chain of reactions in the photosynthetic process, we are not in a position to say much at present.

Similar responses are found in the case of wheat and linseed leaves (tables XIV, XV).

In these cases the optimum lies near 28° C., a lower temperature than that for sugar cane, probably owing to the comparatively cooler season in which flax grows and the consequent adaptability of the material to temperature.

## Discussion

In the preceding pages we have briefly presented a quantitative study of assimilation under various light intensities and carbon dioxide concentration in the light of limiting factors and also of the relative minimum theories. How far these two conceptions hold good under different conditions we shall now discuss.

### LIMITATIONS OF LAW OF LIMITING FACTORS

The essence of this law is that only one factor, that which is slowest, governs the rate of the process, which can be accelerated only by increasing the intensity of this factor. For example, if the rate of the reaction is limited by $CO_2$, only increase in its concentration will increase the photosynthetic rate. Taking a concrete case, we find that under atmospheric concentrations of 0.05 per cent. $CO_2$ and light intensity of 90 c.p. the rate of assimilation is 1.05 mg. (experiment 2). If we increase the concentration to 0.08 per cent. the rate of assimilation also increases to 1.15 mg. (experiment 3). Under 0.05 per cent. carbon dioxide concentration, therefore, the $CO_2$ is the limiting factor, for by an increase in its concentration is the rate accelerated. Inquiry as to whether an increase in other factors under the same $CO_2$ concentration brings about greater assimilatory activity was made, and it was noted that under this limiting condition of $CO_2$ the rate also could be increased to 1,964 mg. by increasing the light intensity to 1500 c.p. (experiment 9). The readings increase by increasing both the concentration of $CO_2$ and the light intensity.

Several other experiments were conducted to see whether the peculiarity observed at this light intensity and $CO_2$ concentration holds good under other conditions. Taking a few examples, the rate of assimilation at 1500 c.p. and 0.12 per cent. $CO_2$ concentration is 6.1 mg. (experiment 11). When the $CO_2$ concentration was raised to 0.403 per cent. under the same light intensity the rate of assimilation rose to 6.38 mg. (experiment 12). On the other hand, when under approximately similar concentrations of

0.12 per cent. the light intensity was increased to 1875 c.p. the rate rose to 6.29 mg. (experiment 14). Here also we notice a rise in the activity by changing either the $CO_2$ concentration or the light intensity to higher units.

These experiments indicate that it is not merely one factor that determines the rate, as BLACKMAN claims, but that the pace of the reaction is determined by at least two factors. Whether by varying the third, namely, temperature, the rate is increased or not we are not in a position definitely to say.

### LIMITATIONS OF THEORY OF RELATIVE MINIMUM

As opposed to BLACKMAN'S conception, HARDER (**6**) holds that of the two factors, light intensity and $CO_2$ concentration, the one that is present in the minimum governs the pace of the reaction to a greater extent. We have explained the nature of the curves showing the relationship of $CO_2$ concentration and light intensity on the basis of this theory, but to test how far it holds good under different circumstances a number of experiments were conducted.

Experiment 9 shows that the rate of assimilation under 1500 c.p. and 0.036 per cent. $CO_2$ is 1.964 mg. An increase in $CO_2$ concentration to 0.124 per cent., *i.e.*, 3.5 times, increases the photosynthesis to 6.1 mg. (experiment 11), *i.e.*, 3.1 times. In other words, the per unit increase in $CO_2$ concentration increases the rate by 0.88 times. On the other hand, when the light intensity is increased to 1875 c.p. (1.25 times under more or less similar $CO_2$ concentrations) the readings are increased to 3.303 mg. (experiment 13), *i.e.*, 1.63 times. In other words, with per unit increase in light intensity the assimilation rate increases 1.3 times. Thus under similar conditions (0.03 per cent. $CO_2$ concentration) unit increase in $CO_2$ brings about 0.88 times increase and unit increase in light accelerates 1.3 times, showing that the reaction is more controlled by light even at high light intensities of 1500 c.p. Here the $CO_2$ concentration is apparently very small while the light is sufficiently high, and therefore the rate, according to the theory of relative minimum, should be governed by $CO_2$ concentration, an idea contrary to the experimental results obtained.

Several experiments were also carried out to test the applicability of the theory to lower light intensities. Experiment 2 shows the assimilation reading at 90 c.p. and 0.051 per cent. $CO_2$ concentration. The rate of photosynthesis is 1.05 mg. When the $CO_2$ concentration is increased to 0.08 per cent., *i.e.*, 1.58 times, the assimilation increases to 1.15 mg. (experiment 3), or 1.09 times. For unit increase in $CO_2$ concentration, therefore, an increase by 0.69 times is marked in the rate of the reaction. On the other hand, when the light is increased to 1500 c.p., *i.e.*, 16.6 times, the rate under approximately similar concentrations of $CO_2$ is 1.964 mg. (experiment 9),

an increase of 1.87 times. In other words, for unit light increase there is acceleration by 0.11 times only. Under low light intensity, therefore, the rate is controlled by $CO_2$ concentration and not by light intensity.

A third aspect remains to be settled, to determine how far the relative minimum theory holds good at high concentrations of $CO_2$. Several experiments were designed to prove its applicability. Under 375 c.p. and 0.121 per cent. $CO_2$ concentration the rate of assimilation is 2.391 mg. (experiment 7). This is increased to 2.51 mg., *i.e.*, 1.08 times when the $CO_2$ concentration is raised to 0.407 per cent., by 3.36 times. On the other hand, when light is raised to 1500 c.p., *i.e.*, by four times under the same concentration, the rate is 6.1 mg., an increase by 2.55 times. For unit increase in $CO_2$ concentration and light intensity, therefore, there is an increase of 0.32 and 0.637 times respectively. In this case also light is the determining factor.

These observations indicate that the relative minimum theory of HARDER does not hold good under all conditions. In the majority of cases light, whether of low or high intensity, controls the rate of the reaction. The main reaction, namely, the relationship of unit $CO_2$ increase to the rate of assimilation, remains practically the same, the results under low $CO_2$ concentration and high and low intensities of light being 0.88 and 0.69 times. The increase is practically constant under the different conditions mentioned. Under very high light intensity a slight increase is marked, probably connected with the fact that greater energy is available under the circumstances, which leads to a slight increase in the velocity of the photochemical phase.

We have discussed the limitations of the limiting factor concept as well as of the relative minimum. The evidence relating to interaction of factors favors the view that the rate of photosynthesis is controlled neither by one factor alone (as was held by BLACKMAN) nor by one or a set of two factors present in relative minimum (as claimed by HARDER). Most probably each of the factors affecting the rate of photosynthesis has some definite influence on the rate of assimilation, depending upon the intensity of others, the different velocities of the reaction being brought about by changes introduced in the diffusion, photochemical, and chemical stages of the mechanism. Which of these phases is most affected by a particular factor remains to be determined, but since the whole process consists of a series of interlinked reactions, the effect which a change in one factor brings about in a particular phase may induce slight modification in others as well, thus indicating that there cannot be a sharp line of demarcation between the changes produced by one or the other of the factors.

But with all these minor details, the law of limiting factors in its broad sense appears to be of universal applicability and has therefore been a

means of interpreting simply and logically the phenomenon of photosynthesis in its various aspects. To us it seems that any relationship that is traced between the external factors alone, without taking into consideration the internal changes that take place in the mechanism itself, will not hold good under all circumstances. This is well supported by the present observations, and since the actual process is intracellular, the relationship if any has to be sought in the internal concentrations that these factors bring about rather than in the actual external intensities of the factors.

## Summary and conclusions

As a preliminary step toward the analysis of growth in terms of assimilation and respiration, it was considered of importance to study the factors affecting these two processes. The present paper is an attempt to elucidate the relationship between the external factors light, temperature, and carbon dioxide and photosynthesis, special attention being paid to the concentrations and intensities in which they interact under ordinary natural conditions in the subtropics.

The investigations have been mainly confined to wheat, linseed, and sugar cane plants and the rate of assimilation was determined by the continuous current methods of BLACKMAN. The following conclusions are the outcome of the present work:

1. Under low light intensities the curves showing the relationship of $CO_2$ and assimilation are smooth, with no sharp break as observed by BLACKMAN and his coworkers. The stationary phase is either absent or if present extends only to a small range.

2. With higher light intensities also the curves in wheat and linseed are extremely regular, the only difference being that there is a big range of stationary assimilation with increasing concentrations of $CO_2$, the toxic effect not being so easily marked.

3. The concentration of $CO_2$ at which the readings are maximum depends upon light intensity. The higher the light intensity the higher is this optimum. These facts are probably correlated with greater availability of energy under high intensities.

4. Under zero percentage of $CO_2$ the rate of assimilation increases with increasing $CO_2$, until the respiratory output balances the assimilatory intake of $CO_2$, namely, the compensation point is reached.

5. Light requirements of different crops vary, probably owing to the nature of the end products formed.

6. Increase in temperature accelerates the rate of photosynthesis, the rise being the result of the accelerating effect of temperature on the one hand and its depressing effect on the other. The readings show a rise until the former is greater than the latter, an optimum being obtained when the two balance each other.

7. The time effect is earlier at higher temperatures.

8. The optimum in the case of wheat and linseed lies at a lower temperature than in the case of sugar cane, probably owing to the colder climate in which these grow and their consequent adaptability toward temperature.

9. It is not one factor that limits the rate of photosynthesis under any set of conditions; the velocity of the reaction is governed by at least two factors.

10. Whether the $CO_2$ be in high or low concentrations under high intensities of light, the rate is always controlled by light. The theory of relative minimum is limited in application, partially holding good under very low intensities of light and low concentration of $CO_2$.

11. Any relationship that is traced between the external factors alone, without taking into consideration the internal changes that take place in the process as well as the intensities in which the external factors reach the internal tissues, will not hold good under all circumstances.

DEPARTMENT OF PLANT PHYSIOLOGY
INSTITUTE OF AGRICULTURAL RESEARCH
BENARES HINDU UNIVERSITY, INDIA

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# TEMPERATURE AS A PREDETERMINING FACTOR IN THE DEVELOPMENT OF *AVENA SATIVA*[1]

THORA M. PLITT

(WITH THREE FIGURES)

## Introduction

It has been shown that various conditions prevailing during the germination of seeds or during the early seedling stages have more or less pronounced effects on the later development of plants. The most comprehensive summary of such investigations is that of KIDD and WEST (**14**). The effects of different temperatures during the germination period on later plant development have been investigated by a number of workers. APPEL and GASSNER (**1**) reported that both summer and winter cereals were injured if their germination took place at unfavorably high temperatures. GASSNER (**10**) brought out the fact that certain types of oats lodged if they were germinated at 25° C. and then transplanted to the field. Lodging occurred even if the 25° temperature prevailed only during the first two days of germination. In either case indications of lodging appeared about five weeks after germination. Under these conditions the oats also failed to head and consequently to yield any grain. The detrimental effects brought about by high temperature were apparently due to disturbances in the very complex chemical reactions in the plant. These findings are confirmed by those of MAXIMOV (**19**). WALSTER (**24**), as a result of an investigation on the formative effects of high and low temperatures on the growth of barley, suggested that the course of development of barley is to a large extent predetermined at a very early stage in growth by the chemical equilibria within the seedling, especially the carbohydrate-nitrogen ratio.

The present investigation was undertaken with the purpose of obtaining some indications as to what differences, if any, are produced in the germinating grains and in the seedlings of *Avena* because of the different temperature conditions prevailing during germination.

## Part I

## Methods

### A. GROWING THE PLANTS

Kherson oats, Nebraska 21 selection, kindly furnished by DR. W. E. LYNESS of the Agricultural Experiment Station of the University of Ne-

[1] Contribution from the Hull Botanical Laboratory, University of Chicago.

braska, were used. The Nebraska 21 oats were selected in 1907 from a field of Kherson oats originating from seed imported from Kherson, Russia, in 1896; this selection has since been grown continuously at the Station.

The grains were first treated with a 0.25 per cent. solution of uspulun for about 20 minutes and then placed on porous porcelain plates in covered glass bowls. Water was placed in the bowls to reach part way up the sides of the plates, and more added later as needed. The bowls were then placed in a refrigerator at 5° or in a Minnesota type germinator at 25° C. according to the condition desired. These two temperatures were chosen because they had been used by GASSNER (10) in his investigation of grains.

At the end of five days the entire grains were dried with absorbent paper, weighed, and placed in sufficient hot 95 per cent. alcohol to make a final extract of 80 per cent. alcohol. About 0.5 gm. of calcium carbonate was added to counteract any acidity in the sample. The whole was heated for about 30 minutes on the steam bath to stop enzymatic action, and set aside for a few days. The extract was then poured off and the grains ground in a mortar before reextraction with 80 per cent. alcohol.

In order to investigate the seedlings, the grains were first treated with 0.25 per cent. uspulun for about 20 minutes and then placed on moist cellucotton in petri dishes. After having been five days in the germinator or the refrigerator, the seedlings were planted in boxes built to fit into glass chambers, about $100 \times 90 \times 25$ cm. These boxes were filled with good garden soil. In these chambers both the temperature and the humidity were controlled. The treatments given the plants are shown in table I.

When the seedlings were three weeks old from the time they were first placed in the refrigerator or the germinator, the tops were cut off at the soil line, separated into two samples, weighed, and treated as previously discussed for the seeds.

The grains in the resting condition were ground in an Enterprise mill, weighed, and extraction carried on as with the seeds. In this and in all

TABLE I

TREATMENT AND GROWING CONDITION OF OATS

| LOTS | GERMINATION TEMPERATURE FOR FIRST 5 DAYS | SUBSEQUENT GROWING TEMPERATURE FOR 16 DAYS | PERCENTAGE RELATIVE HUMIDITY DURING THESE 16 DAYS |
|---|---|---|---|
| | °C. | °C. | % |
| A | 5 | 15.0 | 70 |
| AA | | | |
| B | 25 | 26.4 | 70 |
| C | 25 | 26.4 | 40 |
| D | 25 | 15.5 | 70 |
| E | 5 | 25.0 | 70 |

the above cases, the second extract gave no reaction for reducing sugars, so extraction was deemed to be complete.

### B. Chemical analysis

Dry weight.—The percentage dry weight was obtained by heating the plant material in an oven at 103° C. for 30 minutes to stop enzymatic action, then at about 70° C. in a Cenco electric vacuum oven until constant weight was attained.

Soluble carbohydrates.—The 80 per cent. extract was prepared for the soluble carbohydrate determinations by the tentative method XII, 37, (a) of the Methods of Analysis (**2**). Potassium oxalate was used to clear the solution of excess lead acetate as advocated by Loomis (**16**). Inversion was accomplished by the official, first action, method for sucrose XXVII, 22 of the Methods of Analysis (**2**). The resulting reducing sugars were determined by the Kertesz variation of the Bertrand method (**13**). The soluble carbohydrates are calculated as glucose.

Hydrolyzable carbohydrates.—Acid hydrolyzable carbohydrates were determined upon samples taken from the dried and weighed residue left from the alcoholic extract. About 2 gm. of residue were accurately weighed and placed in flasks fitted with reflux condensers. Two hundred cc. of water and 12.5 cc. of concentrated hydrochloric acid were added and the whole boiled gently for three hours. The resulting solution containing the hydrolyzed products was filtered off and the wash water of the residue included with it. This was made up to 500 cc. An aliquot was neutralized with powdered sodium carbonate and the reducing sugars determined by the Kertesz variation of the Bertrand method (**13**). The acid hydrolyzable carbohydrates are calculated as glucose.

Soluble nitrogen.—The soluble nitrogen was determined from the alcoholic extract by the method recommended by Pucher, Leavenworth, and Vickery (**22**) with the variations of T. G. Phillips. These consisted in the use of copper sulphate crystals instead of mercury as a catalytic agent, and the omission of the use of potassium permanganate and of sodium thiosulphate at the completion of the digestion.

Insoluble nitrogen.—Insoluble nitrogen was determined from the residue remaining from the alcoholic extract by means of the official method of Gunning, II, 22 of the Methods of Analysis (**2**).

Total nitrogen is obtained by the addition of soluble and insoluble nitrogen. Likewise total carbohydrates are obtained by the addition of soluble and hydrolyzable carbohydrates.

### C. Microchemical examination

The methods used microchemically are as follows:

Pectin.—Ruthenium red (1:10,000): red stain (**8**).

STARCH.—Iodine potassium iodide: indigo. Chloral hydrate added to clear the tissues if the starch grains were small (**8**).

REDUCING SUGARS.—Modified Fehling test of cupric tartrate and 10 per cent. sodium hydroxide, heated at about 40° C. for about two minutes (**8**).

SILICA.—Phenol crystals heated to dissolve them, then clove oil added to prevent recrystallization: pink sheen on silica deposits. The silica method is given in detail by FROHNMEYER (**9**).

PROTEINS AND AMINO ACIDS.—Xanthoproteic reaction: yellow-orange (**8**). Biuret reaction: blue to violet (**8**). Millon's reagent: red (modified by BENSLEY **4**). Ninhydrin: blue. The solution (0.1 gm. ninhydrin to 10 cc. water) is added to the section and left standing for one or two hours. LOEW (**15**) has reported on the use of this reagent.

ALEURONE GRAINS.—Sections are placed in alcohol-ether for 15 minutes to remove fats, then left in borax carmine for about two hours.

HEMICELLULOSE.—Congo red: red stain in walls (**21**). Chlorzinc iodide: blue stain in walls (**21**). Solubility in hot 5 per cent. hydrochloric acid (**8**). Polarizing microscope: walls anisotropic (**21**).

## Results

### A. GERMINATION

Grains germinated at 5° for five days have a markedly higher percentage of dry weight than grains germinated at 25° C. for five days (table III). Their percentage dry weight based on the original weight of the grains is also higher (table III). There is no question as to the greater imbibition of water by the grains and seedlings at the higher temperature, as is evident from table II. The greater amount of water imbibed is probably due to the fact that the seedlings at 25° were in a more advanced stage of development than the seedlings at 5° C. The seedlings at the higher temperature were in fact 2 to 3 cm. high, whereas those at the lower temperature showed the hypocotyls just breaking through.

TABLE II

INCREASE IN FRESH WEIGHT OF GRAINS WHEN GERMINATED AT 5° AND AT 25° C. FOR 5 DAYS

| LOTS | GERMINATION TEMPERATURE | ORIGINAL WEIGHT | WEIGHT AFTER 5 DAYS OF GERMINATION |
|---|---|---|---|
| | °C. | gm. | gm. |
| G | 5 | 100 | 178 |
| H | 25 | 100 | 248 |

Germination at the lower temperature resulted in a somewhat higher percentage of hydrolyzable carbohydrates remaining in the grains, as is shown in the calculations based on the original weight of the grains before germination (table III). The higher percentage of hydrolyzable carbohydrates is probably due to the fact that starch hydrolysis proceeds more slowly at low temperatures than at high temperatures. Moreover at the higher temperature the more rapid respiration results in a greater consumption of carbohydrates. The total nitrogen does not, of course, differ materially. Thus the proportion of carbohydrates to nitrogen is somewhat higher at the lower germination temperature. The proportion of soluble to insoluble nitrogen is slightly lower under these conditions.

### B. Microchemistry

There are several differences evident in seedlings germinated at the different temperatures. At low temperatures some starch is deposited as very small grains in the parenchymatous tissues of the coleoptile, the coleorhiza, the scutellum, the cortex of the root, and the root cap. At the higher temperature, however, starch is not deposited in the seedling, or if so, only a little which tends to disappear soon. In the resting grain there is no starch in the embryo.

Another difference is in the amount of pectin produced. At low temperature there is pectin all through the coleoptile and the coleorhiza. It is especially abundant in the epithelium of the scutellum. There is some pectin present in the epiblast and in the root cap. At the higher temperature pectin is present in similar amounts in the coleoptile, the coleorhiza, the epithelium of the scutellum, and the epiblast. But there was a great deal more pectin in the root cap and in addition there was considerable pectin in the epidermis of the root. The seedlings which at this higher temperature had attained a height of about 2 cm. exhibited a great deal of pectin in the cell walls of the coleoptile. Pectin was especially prominent in all of the cell walls of the bundles of the coleoptile except the lignified xylem elements. Reducing sugars could not be detected in the embryo of the resting grain. In the grains germinating at 5° C. reducing sugars could not be detected until the third day; even then there were only traces present which could not be localized satisfactorily. By the fifth day there was not much more in evidence. On the other hand, in grains germinating at 25° C. traces of sugar could be detected after one day. On the second day it was found present in the parenchyma of the root and coleoptile.

The majority of the seedlings germinated for five days at 25° C. were 2 to 3 cm. high, as stated previously. These seedlings were high in reducing sugars, particularly in the cortical parenchyma and the epidermis of the stem; some appeared also in the primary leaves. This confirms the

## TABLE III

ANALYSIS OF GRAINS IN THE RESTING CONDITION AND AFTER GERMINATION FOR 5 DAYS AT 5° AND AT 25° C. CALCULATIONS MADE ON BASIS OF DRY WEIGHT (UPPER PART OF TABLE), ON BASIS OF FRESH WEIGHT (MIDDLE PART OF TABLE), AND ON BASIS OF ORIGINAL WEIGHT BEFORE GERMINATION (LOWER PART OF TABLE)

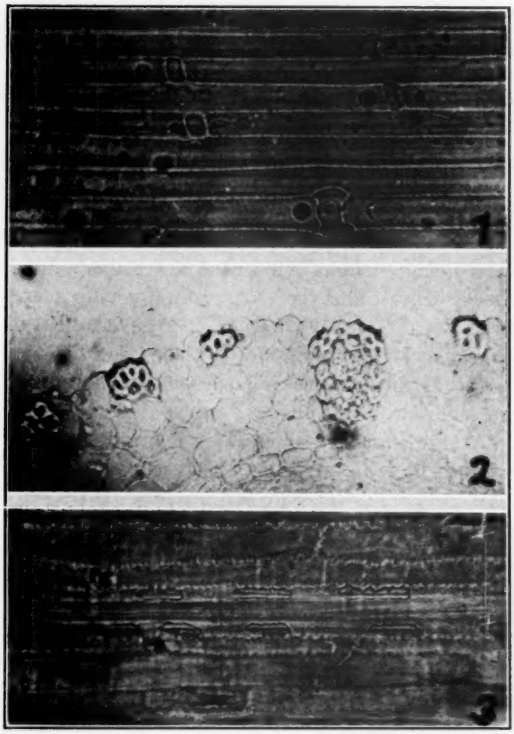
| | Condition | Dry weight | Soluble carbohydrates | Hydrolyzable carbohydrates | Total carbohydrates | Hydrolyzable CH / Soluble CH | Soluble nitrogen | Insoluble nitrogen | Total nitrogen | Insoluble N / Soluble N | Carbohydrates / Nitrogen |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | % | % | % | % | | % | % | % | | |
| F | Resting | 91.9 | 1.528 | 60.92 | 62.45 | 100/2.5 | 0.071 | 1.683 | 1.754 | 100/4.2 | 36.77 |
| G | 5° C. 5 days | 53.39 | 0.205 | 54.67 | 54.88 | 100/0.4 | 0.101 | 1.801 | 1.902 | 100/5.6 | 28.85 |
| H | 25° C. 5 days | 37.86 | 2.428 | 52.53 | 54.96 | 100/4.6 | 0.120 | 1.901 | 2.021 | 100/7.9 | 27.19 |
| F | Resting | 91.9 | 1.404 | 56.00 | 57.40 | 100/2.5 | 0.065 | 1.547 | 1.612 | 100/4.2 | 36.43 |
| G | 5° C. 5 days | 53.39 | 0.110 | 29.19 | 29.30 | 100/0.4 | 0.054 | 0.962 | 1.016 | 100/5.6 | 28.73 |
| H | 25° C. 5 days | 37.86 | 0.926 | 19.89 | 20.82 | 100/4.6 | 0.057 | 0.720 | 0.777 | 100/7.9 | 26.79 |
| G | 5° C. 5 days | 95.04 | 0.195 | 51.96 | 52.16 | 100/0.4 | 0.096 | 1.712 | 1.808 | 100/5.6 | 28.84 |
| H | 25° C. 5 days | 90.86 | 2.207 | 47.73 | 49.94 | 100/4.6 | 0.137 | 1.727 | 1.864 | 100/7.9 | 26.79 |

macrochemical findings of a considerably higher percentage of soluble sugars when germinated at 25° than when germinated at 5° C. (table III).

The protein and amino acid tests, xanthoproteic, biuret, Millon, and ninhydrin, indicated a more rapid mobilization of these substances when germination took place at 25° than at 5° C. In the resting grain all these stains were positive in the embryo and the aleurone layer. The layer of large cells immediately below the aleurone layer was stained particularly with Millon's reagent. In the aleurone layer there are present many aleurone grains (borax carmine method); in these aleurone grains there are no inclusions. During germination at the lower temperature the growing point, the primary leaves, and the root gradually take deeper stains. The ninhydrin test shows the most change. Whereas in the resting grain only a faint blue rim of color is obtained about the edge of the cover glass, as germination proceeds the color becomes deeper and localized in the regions just mentioned; moreover this reagent seems to stain the vascular tissues particularly. At the higher germination temperature after one day the primary leaves and the root stand out. After two days the biuret reaction is purple in the growing point and the primary leaves, but bluish in the coleoptile and the root (especially the stele), possibly indicating differences in the protein units present. After three days in the germinator ninhydrin yields a deep blue color in the root tip, the stele of the root, the primary leaves, particularly in the vascular bundles, and in the bundles of the coleoptile. These reactions continue through the germination period.

It was noted that in the resting grain the walls of practically all parenchyma cells are irregularly thickened, particularly at the corners. These thickenings are stained blue by chlorzinc iodide and red by Congo red. They are soluble when the sections were heated to the boiling point in 5 per cent. hydrochloric acid. Examination of the sections with the polarizing microscope before treating with acid showed these thickenings to be anisotropic; but after the acid treatment this quality was lost. As growth proceeds these thickenings decrease slightly in the original regions but appear to become more prominent in the parenchyma of the leaves. This may be an instance of hemicellulose cell wall thickenings, possibly functioning as a reserve food.

Some 6-weeks old plants were examined for the distribution of silica. These plants had been germinated at 5° or at 25° C. for five days and had then been transplanted to a plot of soil in the greenhouse. Of the plants that were germinated at 5° C. individuals were examined which stood erect. The silica was deposited mainly in the walls of the short epidermal cells of the stem (fig. 1). There was also some deposition in the cell walls of the stomata. The irregular occurrence of circular dark spots in the walls of the long epidermal cells of the stem also represent silica deposited in the




FIGS. 1–3. Fig. 1, epidermis of stem of 6-weeks old oat plants showing silicon deposit in cell walls of short cells particularly, some in stomatal cell walls, and traces in circular areas in long cells. Fig. 2, cross section of a leaf sheath of a 6-weeks old oat plant with silicon deposited in certain epidermal cells over the bundles. Fig. 3, epidermis of a leaf sheath of a 6-weeks old oat plant with silicon deposited in separate cells arranged in rows.

cell walls as a fine network. Figure 2 shows the cross section of a leaf sheath, and figure 3 the longitudinal view. It will be noted that the silica is deposited in epidermal cells over the bundles, but that these cells as seen in top view are separated from one another by cells whose walls do not contain silica. A few of those plants which were germinated at 25° C. had lodged. These were examined for silica, but no differences were found between them and the erect plants.

### C. After three weeks

The two series in the controlled chambers were run three weeks apart, but in immediate succession: May 5 to 26 and May 24 to June 15, 1931.

## TABLE IV

ANALYSIS OF PLANTS 3 WEEKS OLD GROWN AT 15.5° C. (FIRST AND THIRD PARTS OF TABLE) AND AT 25°–26.4° C. (SECOND AND FOURTH PARTS OF TABLE). UPPER HALF OF TABLE CALCULATED ON DRY WEIGHT BASIS; LOWER HALF ON FRESH WEIGHT BASIS

| | Growing temperature | Relative humidity | Germination Temperature | Dates | Dry weight | Soluble carbohydrates | Hydrolyzable carbohydrates | Total carbohydrates | Hydrolyzable CH / Soluble CH | Soluble N | Insoluble N | Total N | Insoluble N / Soluble N | Carbohydrates / Nitrogen | Height |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | °C. | % | °C. | | % | % | % | % | | % | % | % | | | cm. |
| A | 15.5 | 70 | 5 | 5/5–5/26 | 10.55 | 2.65 | 9.09 | 11.74 | 100/29.1 | 0.846 | 5.591 | 6.437 | 100/1.5 | 1.83 | 11.4 |
| AA | 15.5 | 70 | 5 | 5/24–6/15 | 11.36 | 6.00 | 12.10 | 18.10 | 100/49.9 | 0.917 | 5.450 | 6.367 | 100/16.8 | 2.84 | 11.6 |
| D | 15.5 | 70 | 25 | 5/24–6/15 | 12.12 | 14.60 | 18.57 | 33.17 | 100/78.6 | 1.464 | 8.682 | 10.146 | 100/16.9 | 3.28 | 11.8 |
| B | 26.4 | 70 | 25 | 5/5–5/26 | 12.36 | 8.33 | 12.14 | 20.47 | 100/68.6 | 0.760 | 4.800 | 5.560 | 100/15.8 | 3.68 | 19.6 |
| E | 25 | 70 | 5 | 5/24–6/15 | 11.91 | 5.61 | 12.37 | 17.99 | 100/45.4 | 1.115 | 4.363 | 5.478 | 100/25.6 | 3.78 | 18 |
| A | 15.5 | 70 | 5 | 5/5–5/26 | 10.55 | 0.279 | 0.959 | 1.238 | 100/29.1 | 0.090 | 0.611 | 0.701 | 100/15.1 | 1.78 | 11.4 |
| AA | 15.5 | 70 | 5 | 5/24–6/15 | 11.36 | 0.676 | 1.375 | 2.051 | 100/49.9 | 0.105 | 0.619 | 0.724 | 100/16.8 | 2.66 | 11.6 |
| D | 15.5 | 70 | 25 | 5/24–6/15 | 12.12 | 1.769 | 2.250 | 4.019 | 100/78.6 | 0.198 | 1.052 | 1.250 | 100/16.9 | 3.23 | 11.8 |
| B | 26.4 | 70 | 25 | 5/5–5/26 | 12.36 | 1.030 | 1.501 | 2.531 | 100/68.6 | 0.094 | 0.594 | 0.688 | 100/15.8 | 3.68 | 19.6 |
| E | 25 | 70 | 5 | 5/24–6/15 | 11.91 | 0.668 | 1.475 | 2.143 | 100/45.3 | 0.133 | 0.520 | 0.655 | 100/25.6 | 3.28 | 18.2 |

The increased day length in the second period increased all of the carbohydrate fractions, and did not cause any appreciable change in the nitrogen fractions (table IV, A and AA). Notwithstanding this increase in carbohydrates the two series are comparable, for the results show the same trend. Thus the proportion of carbohydrates to nitrogen is less in both A and AA, germinated at low temperature, than in B or D, germinated at the higher temperature.

At the end of three weeks the seedlings grown at about 25° (table IV, B and E) were the taller regardless of the other conditions applied in these experiments. Seedlings germinated at 25° (table IV, B and D as compared with AA and E) exhibit a higher percentage of dry matter than those germinated at 5° C., also higher percentages of soluble carbohydrates and generally a higher percentage of total carbohydrates. There is a decidedly higher proportion of soluble to hydrolyzable carbohydrates: 68.6 : 100 and 78.6 : 100 (B, D) as compared with 49.9 : 100 and 45.4 : 100 (AA, E).

Those plants which were germinated at 25° C. and grown at about the same temperature (table V, B) were higher in all the carbohydrate fractions and slightly lower in all the nitrogen fractions than those germinated at 5° and grown at 15° C. (table V, A). The total carbohydrates are 8 per cent. higher and total nitrogen is about 0.9 per cent. lower; hence their proportion of carbohydrates to nitrogen is decidedly higher. The proportion of soluble to hydrolyzable carbohydrates is also far higher than with the other treatment; 68.6 : 100 (B) at about 25° C. compared with 29.1 : 100 at low germination and growth temperatures (A).

At this age (three weeks) the proportions of soluble to insoluble nitrogen did not seem to be correlated with any specific factor. For instance, the proportion of insoluble to soluble nitrogen is 100 : 25.6 when germination took place at 5° and growth at 25° C. (table V, E); but another lot (AA) germinated at 5° and grown at 15.5° C. has a proportion of 100 : 16.8, and yet another lot (B) germinated at 25° and grown at 25° C. has a proportion of 100 : 15.8.

One lot of oats was germinated at 25° C. and grown at the same temperature but at a relative humidity of 40 per cent. (table VI, C) instead of 70 per cent. (B). These plants did not produce so rank a growth as the plants grown at a humidity of 70 per cent., as may be seen from the measurements of their heights (table VI); and their percentage of dry matter was slightly higher. The proportion of soluble carbohydrates to hydrolyzable carbohydrates is slightly less. Since the percentage of total carbohydrates is less but the percentage of nitrogen about the same, the ratio of carbohydrates to nitrogen is slightly less when the humidity is decreased.

## Discussion

The greater imbibition of water and the greater growth of the seedlings at the end of the 5-day germination period with a temperature of 25° as compared with the behavior of seedlings grown for the same length of time at 5° C. is in agreement with the general findings as summed up by BARTON-WRIGHT (3). He states that not only does the temperature affect the rate of entry of water into the seed as well as the growth rate of the radicle, but it also affects the resistance of the seed coat to the extrusion of the radicle. *Phaseolus vulgaris* shows evidence of germination at 9° C., and the growth rate increases with rising temperatures to 36° and ceases at 46° C. Minimum, optimum, and maximum germination temperatures for rye are 1°, 25°, and 36° C. respectively. The optimum points are somewhat dependent on the time factor.

The results obtained concerning the amount of carbohydrates present in the low temperature seedlings are in agreement with the findings of DICKSON, ECKERSON, and LINK (7) that 10-day old wheat seedlings grown at low soil temperatures are practically high carbohydrate plants.

It is well known that plants respire more rapidly at higher temperatures than at lower ones. This fact is reflected in the percentage of dry weights obtained at the end of the first five days of germination at different temperatures. DAY (6), in working with barley, measured the carbon dioxide output for ten days at three different temperatures. He found that the average output per hour was greater at 60° F. than at 38°–43°, and still greater at 70°. Another feature was that the respiration increases to a maximum and then decreases again. At the lowest temperature given, this maximum occurs on the fourth and fifth days. But at the highest temperature it is shifted to the third day.

It seems likely that the greater amount of soluble sugar present in oat grains germinated for five days at 25° C. is associated with the fact that no starch is deposited in the embryo under those circumstances, whereas starch is deposited at the lower temperatures usually considered more favorable for the growth and development of this plant. In this respect the behavior of oats is similar to that of barley as reported by BROWN and MORRIS (5).

Increase in pectin materials at high germination temperature in oats parallels the results obtained in wheat (7).

Lower germination temperatures are generally recommended as being more favorable for growth and development than higher ones. The different rates of hydrolysis of storage materials and of synthesis of new materials at higher temperatures as compared with lower ones may conceivably affect the later development of plants, directly or indirectly. Thus at the lower temperature the starch in the endosperm is hydrolyzed more rapidly than the protein; also there is less vegetative growth than at the higher

## TABLE V

ANALYSIS OF PLANTS 3 WEEKS OLD GERMINATED AT 5° (UPPER PORTION OF TABLE) AND AT 25° C. (LOWER PORTION OF TABLE); CALCULATED ON DRY WEIGHT BASIS

| | Germination temperature | Growing temperature | Relative humidity | Dates | Dry weight | Soluble carbohydrates | Hydrolyzable carbohydrates | Total carbohydrates | Hydrolyzable CH / Soluble CH | Soluble nitrogen | Insoluble nitrogen | Total nitrogen | Insoluble N / Soluble N | Carbohydrates / Nitrogen | Height |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | °C. | °C. | % | | % | % | % | % | | % | % | % | | | cm. |
| | | | | 5/5 | | | | | 100 | | | | 100 | | |
| A | 5 | 15.5 | 70 | 5/26 | 10.55 | 2.65 | 9.09 | 11.74 | 29.1 | 0.846 | 5.591 | 6.437 | 15.1 | 1.83 | 11.4 |
| | | | | 5/24 | | | | | 100 | | | | 100 | | |
| AA | 5 | 15.5 | 70 | 6/15 | 11.36 | 6.00 | 12.10 | 18.10 | 49.9 | 0.917 | 5.450 | 6.367 | 16.8 | 2.84 | 11.6 |
| | | | | 5/24 | | | | | 100 | | | | 100 | | |
| E | 5 | 25.0 | 70 | 6/15 | 11.91 | 5.61 | 12.37 | 17.99 | 45.4 | 1.115 | 4.363 | 5.478 | 25.6 | 3.78 | 18.2 |
| | | | | 5/5 | | | | | 100 | | | | 100 | | |
| B | 25 | 26.4 | 70 | 5/26 | 12.36 | 8.33 | 12.14 | 20.47 | 68.6 | 0.760 | 4.800 | 5.560 | 15.8 | 3.68 | 19.6 |
| | | | | 5/24 | | | | | 100 | | | | 100 | | |
| D | 25 | 15.5 | 70 | 6/15 | 12.12 | 14.60 | 18.57 | 33.17 | 78.6 | 1.464 | 8.682 | 10.146 | 16.9 | 3.28 | 11.8 |

## TABLE VI

ANALYSIS OF PLANTS 3 WEEKS OLD, GERMINATED AT 25°, GROWN AT 26.4° C., BUT AT DIFFERENT RELATIVE HUMIDITIES: 40 AND 70 PER CENT. CALCULATED ON BASIS OF DRY WEIGHTS (UPPER PART OF TABLE) AND ON FRESH WEIGHT BASIS (LOWER PART OF TABLE)

| | Relative humidity | Growing temperature | Germination temperature | Dates | Dry weight | Soluble carbohydrates | Hydrolyzable carbohydrates | Total carbohydrates | $\frac{\text{Hydrolyzable CH}}{\text{Soluble CH}}$ | Soluble nitrogen | Insoluble nitrogen | Total nitrogen | $\frac{\text{Insoluble N}}{\text{Soluble N}}$ | $\frac{\text{Carbohydrates}}{\text{Nitrogen}}$ | Height |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | °C. | % | | % | % | % | % | | % | % | % | | | *cm.* |
| | | | | 5/5 | | | | | $\frac{100}{}$ | | | | $\frac{100}{}$ | | |
| B | 70 | 26.4 | 25 | 5/26 | 12.36 | 8.33 | 12.14 | 20.47 | $\frac{}{68.6}$ | 0.760 | 4.800 | 5.560 | $\frac{}{15.8}$ | 3.68 | 19.6 |
| | | | | 5/5 | | | | | $\frac{100}{}$ | | | | $\frac{100}{}$ | | |
| C | 40 | 26.4 | 25 | 5/26 | 13.74 | 5.73 | 10.67 | 16.40 | $\frac{}{53.7}$ | 0.840 | 4.629 | 5.469 | $\frac{}{18.1}$ | 3.00 | 14.1 |
| | | | | 5/5 | | | | | $\frac{100}{}$ | | | | $\frac{100}{}$ | | |
| B | 70 | 26.4 | 25 | 5/26 | 12.36 | 1.030 | 1.501 | 2.531 | $\frac{}{68.6}$ | 0.094 | 0.594 | 0.688 | $\frac{}{15.8}$ | 3.68 | 19.6 |
| | | | | 5/5 | | | | | $\frac{100}{}$ | | | | $\frac{100}{}$ | | |
| C | 40 | 26.4 | 25 | 5/26 | 13.74 | 0.787 | 1.466 | 2.253 | $\frac{}{53.7}$ | 0.118 | 0.633 | 0.751 | $\frac{}{18.1}$ | 3.01 | 14.1 |

temperature (**7**). In the present investigation both the macrochemical and the microchemical data seem to indicate that the rates of carbohydrate and protein hydrolyses and utilization are definitely affected by an increase in germination temperature. ZALESKI (**26**) reports that as the protein in *Lupinus augustifolius* is broken down, much asparagin appears. The amount of asparagin obtained increases with rise in temperature. The temperature coefficient follows the VAN'T HOFF rule, $Q_{10} = 2.5$. The indications are that the slower rate of hydrolysis of protein at lower temperatures in oats makes for conditions of growth more favorable to the complete development of the plant.

The most outstanding characteristic of the 3-weeks old plants which had been germinated at 25° C., regardless of the subsequent growing conditions here considered, was the marked increase of total carbohydrates in the tops of the plants over those germinated at 5° C. These high temperature plants had already reached the stage at which photosynthetic activity might begin, at the time the others were just breaking through the seed coats. GREGORY (**12**) found in *Cucumis sativus* that the growth rate of leaf surface increases with the temperature, and that it is dependent on the area of the leaf surface already present. But on investigating the relative rate of increase (rate of increase per unit of leaf surface already present) he found it to be independent of the temperature. He states that the differences in the final leaf area between plants grown at different temperatures must therefore be related to the time elapsing from germination before the photochemical process can begin, *i.e.*, the time elapsing before expansion of the foliage leaves begins. This is a matter of growth and differentiation in the apex and has what he calls a normal temperature coefficient, $Q_{10} = 2.5$. Thus the delay in the development of the first leaf is determined by the temperature at which the developmental processes occur. He believed that a nitrogenous leaf-forming substance is involved. Thus any later differences in leaf surface are caused by the speed with which the plant attained its first leaf. The present experiment seems to point to the conclusion that, since the seedlings at the higher temperature were further developed when transplanted from the 25° germinator to the chambers than those transplanted from the 5° C. refrigerator, they had the advantage of an earlier start in photosynthetic activity which expressed itself in a higher percentage of carbohydrates in the tops of the plants at the age of three weeks.

Analyses of older plants, such as those of clover by TOTTINGHAM (**23**), indicate a higher percentage of polysaccharides in plants grown at 16.9° to 23.3° C. than in those grown at 23.2° to 28.2° C. It may be a complicating factor that in this experiment he collected the plants grown at the higher temperature two weeks sooner than the others, in order that all might have

attained the same size for analysis. Yet he again obtained in buckwheat grown at 16.0 to 19° C. a percentage of polysaccharides higher than when grown at 20.5° to 25° C., with all of them harvested at the end of ten weeks. He attributes the decrease mainly to the impossibility of polysaccharide storage at higher temperatures because of greater consumption of sugars with increased respiration. On the other hand it is probable that different optima exist for different stages of development of a plant. GASSNER (**10**) recommends for Uruguay oats a low temperature germination followed by a rapid rise in temperature in order to achieve maturity as early as possible.

Furthermore MCLEAN (**20**), in investigating effects of climatic conditions, using soy beans, came to the conclusion that temperature was the limiting condition for growth during the first two weeks in practically all cases. During the second two weeks of growth, however, with exactly the same environmental conditions, the moisture relation (rainfall-evaporation ratio) appears to have been the limiting condition, especially if the temperature was high. This must be due to a difference in the internal conditions of the plants at the different developmental stages. Growth during the early period consisted largely in stem elongation, which must have been accomplished at the expense of material stored in the seed. The rate of development of the plants was therefore probably dependent on the rate of hydrolysis of storage materials and of translocation from the cotyledons to the growing points, and hence dependent on the temperature. On the other hand, during the second two weeks while leaf expansion occurs, greater transpiration will increase the water requirement. So the moisture relation would be a greater factor than before.

APPEL and GASSNER (**1**) report that various cereals, such as wheat, barley, and oats, raised entirely in the warm greenhouse germinated rapidly and continued to grow rapidly. They had already attained a height of 15 cm. when those in the cool greenhouse germinated. After three weeks, however, the rate of growth of those at the high temperature decreased so that finally they were overtaken by those at low temperature. WALSTER (**24**) noted a similar phenomenon. Barley plants in the warm house, which were several inches high before the plants in the cool house had come up, during the first two weeks maintained a more rapid growth. But at the end of a month the plants in the cool house had outstripped those in the warm house in their growth rate. At the end of six weeks all of the plants in the cool house had outgrown those in the warm house.

Analyses of plants at later stages than those made in this investigation may well yield interesting results.

The findings concerning the highly localized deposition of silica agree with those made by WYSSLING (**25**) in his survey of the distribution of silica in plants. Since the deposition of silica does not result in a continu-

ous structure, and since silica is only a very small part of the mechanical structure of the stem and leaf sheaths, it seems unlikely that it should be a factor in determining an erect or a recumbent habit of the plant. Rather, as set forth by WYSSLING, it seems to be an excretion of a nonessential element absorbed in different quantities under differing conditions.

## Part II

Other varieties of oats were tested for possible responses to different germination temperatures. Through the courtesy of the Agricultural Experiment Station at the University of Tennessee four varieties were obtained, Hatchett, Lee, Turf B, the Fulghum 699–2011. From the State College of Agriculture at the University of Georgia were obtained three other varieties, Fulghum, Norton, and Appler. Each of these varieties was subjected to the following four germination conditions: (*a*) 2° for 5 days; (*b*) 25° for 5 days; (*c*) 2° for 2 days, then 25° for 3 days; (*d*) 25° for 2 days, then 2° for 3 days. Treatment was begun on May 27, 1932. At the close of the 5-day period the germinating grains or seedlings were planted in a plot of soil in the greenhouse. On September 19 the experiment was terminated.

Observations were made on dates of heading and possible evidences of lodging. In this series there appeared to be no clear correlation between the temperatures prevailing during germination and dates of heading (these dates were late in the season for oats). Dates of heading differed within a single variety, but no general trend was observable when comparing the different varieties. As for evidences of lodging, there was no bending of the culms in the lower nodes or internodes. In a few cases the culms were leaning over or even broken, but this appeared to be the result of the method of watering. At times the temperature of the greenhouse rose considerably, even reaching 124° F. (51.1° C.). It seems obvious that these varieties of oats do not give a lodging response to high temperatures applied either early or late in the life of the plants.

## Part III

In order to approximate more closely the experimental material reported by GASSNER (**10**), some South American varieties of oats were tested. These were secured from Dr. ALBERTO BOERGER, Director of the Agricultural Experiment Station at Montevideo through the courtesy of Mr. JOSE RICHLING, Consul General of Uruguay in New York. These oats are classified as follows: 1095a belongs botanically to the species *Avena byzantina;* BID belongs to the species *A. satia,* originating from a hybrid of two forms of this species; 64s, *A. capa,* represents a type native of Uruguay and belongs to the species *A. sativa.* GASSNER reports using "*Avena* del pais"

or native Uruguay oats. The seeds falling to the ground in the field make pastures which are good for several years. BOERGER states that although none of these samples was harvested in fallow land, it would be perfectly feasible to obtain them from such land owing to the fact that fallen seeds of any kind soon become transformed into what are called "avena gaucha" (wild oats) offering valuable pasture for winter and fall seasons.

The investigation of the response of these oats to germination temperatures was undertaken in the laboratories of the Botany Department at Columbia University through the courtesy of Professor SAM F. TRELEASE. The oats were treated as previously, namely, germinated at 25° and 5° C. for five days and then planted in the greenhouse. Treatment of the grains was begun on March 25, 1933, and the plants were harvested on June 8.

No lodging was observed in any of these plants.

One lot of the oats headed, namely, the 64s oats which were germinated at low temperature (5° C.). All were bearing grain when harvested. A parallel series, but with germination taking place at 25° C., bore no grain whatsoever. Of the BID oats (5° C. germination) only one culm headed; of the 1095a oats none headed. The varieties are evidently quite distinct from one another, for BOERGER reports the increased yield of the 64s oats. There are also differences in the vegetative portions of the plants: 1095a produces thin culms and fine leaves, BID thicker culms and broader leaves, and 64s the sturdiest plants.

Such a difference in yield was reported by GASSNER (**10**) when working in Uruguay: Uruguay oats kept for the first ten days at 6°–9° C. headed after two months, likewise those kept for ten days at 6°–9° then for two days at 25° C., and also those kept for the first five days at 6°–9° and then for two days at 25° C. But oats germinated at a constant temperature of 25° C. failed to head by the end of eight months; even those which were exposed to a temperature of 25° only the first two days and then at 6°–9° C. for eight days failed to head.

GASSNER (**11**) further differentiates between summer and winter cereals. According to him the latter require a certain period of cold at some time in the life cycle in order eventually to go over into the reproductive phase. The summer cereals, on the other hand, will go over into the reproductive phase even without undergoing such a cold period, although they also appear to be influenced by such an exposure. MAXIMOV (**19**), in testing *Avena byzantina*, found that as the germination temperature was decreased from 26° to 0° C. the vegetative period was shortened from 80 days to 31 days, and simultaneously the yield was increased. The principle of shortening the length of the vegetative stage of grains seems to be applied on a large scale in Russia under the name of Jarovization. The methods em-

ployed and a consideration of the factors involved are presented by LYSSENKO (**17, 18**).

It was thought that higher germination temperatures than 25° C. might cause greater disturbances in the growth of the oat plants. From June 20 to July 24 a number of plantings were made in the greenhouse at the University of Chicago; these were observed until November 24, 1933. The oats were: Kherson oats Nebraska 21 selection, and the oats 1095a, 64s, and BID from Uruguay. The grains were first germinated for five days at 5°, 10°, 25°, 30°, 32.5°, 35°, 37.5°, and 40° C., then planted in soil plots.

The last two temperatures, 37.5° and 40° C., were evidently fatal, for none of the grains placed in these germinators ever germinated. At 35° C. there was a low percentage germination and subsequent growth was limited. The other stands were fairly good; in no case, however, was any lodging observed.

Only one variety headed, namely, the Kherson oats (Nebraska 21 selection). This is very possibly due to the late date of seeding, for during the spring certain of the 64s oats did head. But of these Nebraska 21 oats only those germinated at 10° and 5° C. headed. These were placed in the refrigerators on June 20 and 26 respectively, and they began to head on August 22 and 29. In this respect these oats were similar to the 64s oats in the preceding series. In no case was any lodging observed. All through the growing period many of the plants were stooling, some of the plants having forty or more culms of all sizes at the end of the experiment. The factor operating to produce this unusual result was not determined.

## Summary

1. Oat grains germinated at 25° C. for five days have a markedly lower percentage of dry weight than grains germinated at 5° C. when calculated either on the basis of fresh weight or on the basis of the original weight before germination. These differences are directly related to the rate of growth of the seedlings at the two temperatures, and the amount of carbon and hydrogen consumed in respiration. A somewhat lower percentage of starch remains in the grains when germinated at the higher temperature, and more water is imbibed by the grains than at the lower temperature. The proportion of soluble to insoluble nitrogen is slightly higher at the higher germination temperature.

2. Microchemically it was found that whereas there is no starch in the embryo of oat grains in the resting condition, starch is deposited in the embryo during germination at 5° C.; but little or none is deposited in the embryos germinated at 25° C. At the higher germination temperature there are more pectin and reducing sugars in the seedling than at the lower temperatures.

3. Protein reactions (microchemical) became more intense as germination proceeded, gradually in the case of the lower temperature, much more rapidly at the higher temperature.

4. Cell wall thickenings were found in the parenchyma of the embryo and the early seedling stages of the oats, giving reactions indicating the presence of hemicellulose.

5. Seedlings grown at about 25° C. were the tallest in the series regardless of the germination temperature; they also had the lowest percentages of nitrogen.

6. Seedlings which grew from grains germinated at 25° C. regardless of the later conditions applied in these series exhibited a higher percentage of dry matter than those germinated at 5° C., also higher percentages of carbohydrates, and the nitrogen percentages were lower. There is a higher proportion of soluble to insoluble acid hydrolyzable carbohydrates.

7. Plants germinated and grown at about 25° C. were higher in carbohydrates and slightly lower in nitrogen; also the proportion of soluble to insoluble acid hydrolyzable carbohydrates is far higher than in plants germinated at 5° and grown at 15° C.

8. The proportions of soluble to insoluble nitrogen varied.

9. Oats germinated and grown at 25° C. but at a relative humidity of 40 per cent. as compared with those at 70 per cent. made less growth; their percentage of dry matter was slightly higher, and the percentages of carbohydrates were lower.

10. The greater amount of carbohydrates in plants germinated at 25° as compared with that in plants germinated at 5° C. is believed to be caused by the fact that the seedling development is much further advanced at the time of transplanting, so that photosynthetic activity begins several days earlier.

11. Silica in 6-weeks old plants was found to be deposited in the same manner in both erect and recumbent plants. It is highly localized in certain cells and these cells form no consecutive structure. Hence silica is believed to be a negligible factor in the supporting mechanism of the plant.

12. High temperatures during the very early phases of germination of oats had much less effect on lodging of oats than the work of GASSNER indicates. No temperature treatment during early germination was found that would consistently cause lodging to develop, either immediately or in later life.

Appreciation is expressed to Dr. CHARLES A. SHULL for his interest and guidance, and to Dr. S. V. EATON for his assistance with the microchemical and macrochemical methods. Thanks are also extended to the institutions and individuals who kindly furnished the seeds used for the experiments.

VASSAR COLLEGE
POUGHKEEPSIE, NEW YORK

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# ABSORPTION OF SULPHUR DIOXIDE BY ALFALFA AND ITS RELATION TO LEAF INJURY[1]

MOYER D. THOMAS AND GEO. R. HILL, JR.

(WITH FIVE FIGURES)

## Introduction

A knowledge of the amount of sulphur dioxide which must be absorbed by vegetation in order to produce a definite amount of leaf destruction, rather than the amount to which vegetation may be exposed, is fundamental to an understanding of the smoke problem. It is particularly important to know the relations existing between the rate of absorption and the total quantity of absorption of the gas and the extent of leaf destruction, since it will be shown that sulphur dioxide absorbed in the leaves of alfalfa is rapidly converted into a much less toxic form. Moreover, the rate of absorption in a given plant system varies widely under different environmental conditions. The most important consideration, therefore, is not the concentration of the sulphur dioxide in the air alone, but rather the concentration, the duration of the fumigation, and the rate at which the gas is absorbed by the plant, taken together. Accordingly an exposure which might cause severe injury under one set of conditions might be entirely harmless under another set. In the absence of data showing the rate of absorption it is impossible to say whether the different effects which are produced by a given set of exposure conditions are due to different quantities of gas absorbed or to different degrees of susceptibility.

Earlier work in this field has been concerned primarily with the effect of duration and intensity of exposure to sulphur dioxide in producing injury on vegetation. A study of the absorption of the gas by chemical analysis of the leaves has usually been incidental and apparently carried out to show that the sulphur content of the tissue actually increased owing to the fumigation treatment. The analytical data in the literature, therefore, do not show definitely the amount of absorbed gas which was associated with a definite amount of leaf destruction. STOKLASA states (**4**, p. 156) that 0.175 per cent. $SO_2$ in lupines, 0.379 per cent. in corn, and intermediate amounts in other crops (expressed as percentage of the dry plant tissue) have been observed to exert a harmful influence. These data seem to represent the

[1] Contribution from the Department of Agricultural Research of the American Smelting & Refining Company, Salt Lake City, Utah. Grateful acknowledgment is made to MILTON R. BERNTSON, JOHN N. ABERSOLD, EVAN HARRIS, A. F. BARNEY, IVAN E. BURGOYNE, THOMAS BUNKALL, LYNN BROWN, GEORGE GARDNER, KARL HARRIS, J. RUSSELL SMITH, and HUGH W. PETERSON for their help in carrying forward this work.

order of magnitude of the effect at least for short fumigations. In many cases, however, material for the analyses was not taken immediately before and immediately after the fumigation treatment, in which cases the analytical data are of very doubtful value for indicating the absorption effect. It has been shown in an earlier paper from this laboratory (**8**) that satisfactory absorption data can be obtained either by analyses of the leaves for total sulphur or by continuous analysis (**6, 7, 8**) of the $SO_2$-air mixture before and after contact with the vegetation. In the former method great care is necessary in taking the samples of vegetation. The latter method offers the advantage that it eliminates sampling errors and also shows the progress of the absorption. Its principal disadvantage lies in the necessity of applying a correction because of absorption of the gas on the walls of the fumigation chamber.

In this paper the amounts of absorbed sulphur dioxide which cause different amounts of leaf destruction in alfalfa are considered. Also the relations existing between the duration and intensity of exposure are analyzed with reference to rate of absorption of the gas by the plant and the attendant leaf destruction. Most of the experimental data have been obtained by the method of continuous air analysis.

## Experimental method and results

During the seasons from 1927 to 1931 a considerable number of fumigation experiments were conducted with the idea of studying the rate of absorption of sulphur dioxide by alfalfa and the relation of the quantity of absorbed gas to the leaf destruction. The work of 1927 was of a preliminary nature, but in 1928 and subsequent years quantitative absorption data were obtained. The fumigation apparatus and technique (**1**) and also the analytical methods (**6, 7, 8**) have already been described. Field plots 5 feet square were covered with a gas-tight celluloid-covered cabinet through which a controlled mixture of $SO_2$ and air was passed. The concentrations of sulphur dioxide and the air volumes at both the intake and outlet of the cabinet were determined. The light intensity, temperature, and relative humidity in the cabinet were also measured. A careful estimate was made of the percentage of leaf area destroyed by fumigation on each plant, as described in the preceding paper (**1**). Many representative stems were taken from the field at intervals of 5 to 7 days throughout the growth of the crop, from which the growth curve, and ratio of leaves to stems, as well as leaf area, were found. From this information and the harvest weight of the crop, the weight and area of the leaves at the time of fumigation could be closely approximated. Further, the amount of gas absorbed when the cabinet was placed on bare soil was studied. The absorption by the soil was found to

be appreciable, but when the ground was covered with celluloid only a small amount of absorption could be attributed to the cabinet, unless the relative humidity was more than 80–90 per cent. In the fumigation work, all exposed soil under the plants was covered with celluloid and the absorption data were corrected for the amount of absorption by the cabinet under similar conditions of fumigation.

The experiments for 1928 to 1930, inclusive, are plotted in figure 1, in which the ordinate is the percentage of leaf area destroyed, and the abscissa the amount of sulphur dioxide absorbed, calculated as parts per million of the dry weight of the leaves. The duration of all the fumigations in charts A and B was between 60 and 100 minutes. The data are segregated into four groups with different types of points, according as the plots had full sunlight or were shaded, and according as the relative humidity was more or less than 80 per cent.

In spite of the fact that the individual points of each group in figure 1 are scattered over an appreciable area of the chart, they clearly tend to follow a straight line course, thus showing that the percentage of leaf destruction is in direct proportion to the amount of sulphur dioxide absorbed. If any relationship other than linearity exists between leaf destruction and absorption of sulphur dioxide, it is not evident in figure 1. The equations of the best fit straight lines, found by the method of least squares through the different types of points in figure 1, are as follows:

Curve A: Summarizing fumigations in which the plants were exposed to full sunlight and the relative humidity was less than 80 per cent.:

$$x = 822 + 35.6y \quad \ldots\ldots (1)$$

in which y is percentage of leaf destruction, and x is $SO_2$ absorbed, calculated as parts per million of dry leaf tissue. The equation states that it requires the absorption of 822 p.p.m. $SO_2$ to cause any leaf destruction at all, and the absorption of 4382 p.p.m. to bleach the leaves completely, under the conditions which obtained in these experiments. The coefficient of correlation, r, of this curve is 0.792, and the standard deviation of an individual absorption, $S_x$, is 527 p.p.m. The number of fumigations represented, n, is 131, and their average duration 1.35 hours.

Curve B: Summarizing experiments in which the plants were shaded and the relative humidity was less than 80 per cent.:

$$x = 1003 + 38.5y \quad \ldots\ldots (2)$$

$n = 67$

$r = 0.795$

$S_x = 538$ p.p.m.

Average duration of fumigation = 1.43 hours.

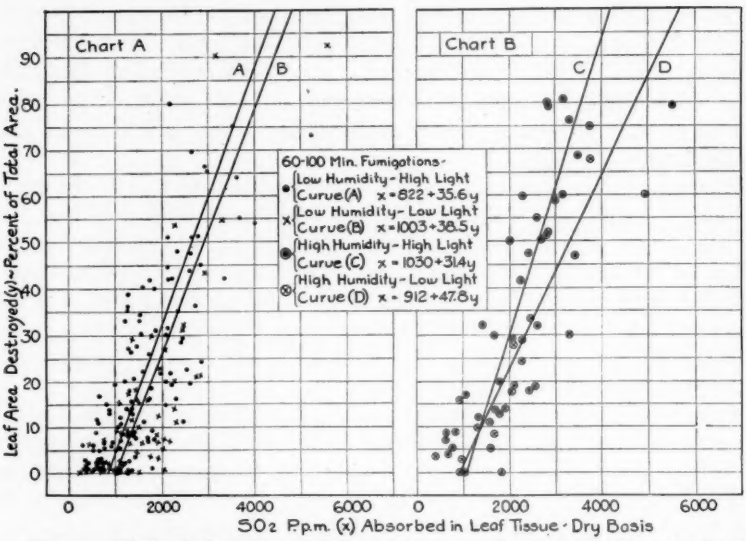


FIG. 1. Effect of absorption of different amounts of sulphur dioxide, calculated as p.p.m. of dry leaf tissue, upon destruction of the leaves of alfalfa under different conditions of light, humidity, and duration of exposure, as indicated in legend. Equations found by method of least squares. 1928–1930 data.

Curve C: Summarizing fumigations in which the plants were exposed to full sunlight and the relative humidity was more than 80 per cent.:

$$x = 1030 + 31.4y \quad \ldots\ldots (3)$$

$n = 29$

$r = 0.817$

$S_x = 581$ p.p.m.

Average duration of fumigation = 1.19 hours.

Curve D: Summarizing fumigations in which the plants were shaded and the relative humidity was more than 80 per cent.:

$$x = 912 + 47.8y \quad \ldots\ldots (4)$$

$n = 25$

$r = 0.864$

$S_x = 536$ p.p.m.

Average duration of fumigation = 1.23 hours.

These equations, as plotted in figure 1, show that there is a tendency for the $SO_2$ absorbed to be slightly less effective in destroying the leaf tissue under conditions of low light, but the differences are not great. This is further confirmed by equations (7) and (8) below. No significance can be

attached to the fact that curves C and D cross each other near the zero ordinate, because of the relatively small number of experiments in each group and their relatively large standard deviations. At very high humidities the absorption by the walls of the cabinet is high, and accordingly the absorption values obtained under these conditions are inherently more uncertain than are the low humidity results. It is likely that when the humidity approaches saturation, particularly in the shade, appreciable amounts of the gas are absorbed on the exterior of the leaves and stems. At low relative humidity, on the other hand, external surface absorption is of minor importance and has probably been allowed for adequately in the "cabinet correction." This is indicated by the fact that in many fumigations, when the plants were inactive, the absorption, by both the plants and the cabinet, exceeded the cabinet correction only slightly.

When all the data in figure 1 are placed in one group, the equation and statistics of the best fit straight line are as follows (curve F, fig. 2):

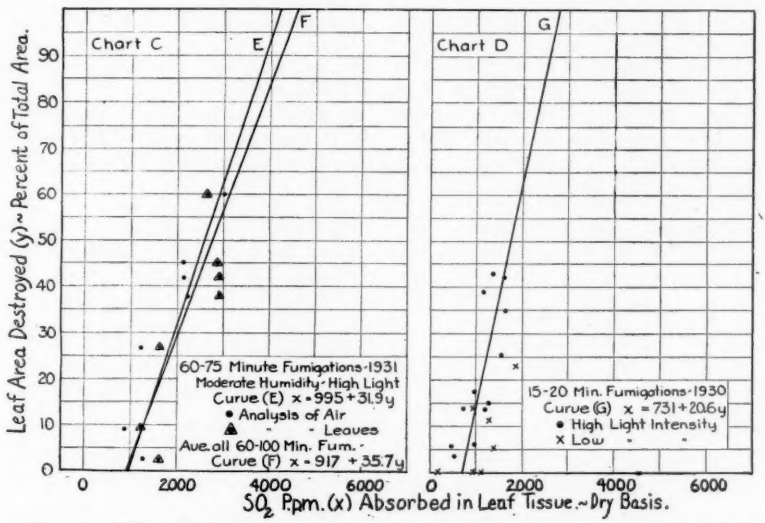


FIG. 2. Effect of absorption of different amounts of sulphur dioxide, calculated as p.p.m. of dry leaf tissue, in different time periods, in causing the destruction of the leaves of alfalfa.

$$x = 917 + 35.7y \qquad (5)$$

$n = 252$

$r = 0.809$

$S_x = 557$ p.p.m.

Average duration of fumigation = 1.34 hours.

This result is only slightly different from that obtained by combining equations (1) and (2):

$$x = 896 + 35.6y \quad \text{.....(6)}$$
$$n = 198$$
$$r = 0.782$$
$$S_x = 541 \text{ p.p.m.}$$
Average duration of fumigation = 1.38 hours.

The coefficients of correlation associated with equations (1) to (6) are all about 0.8, which may be interpreted as indicating a definitely significant degree of correlation. On the other hand, the standard deviations of the absorption values from the curves in the individual experiments are about 550 p.p.m., and therefore an appreciable range of leaf destruction can be expected with the same rate of absorption under different conditions. Of course the dispersion of the data is partly due to the fact that these experiments include treatments of duration ranging from 60 to 100 minutes, which would necessarily imply different rates of absorption, as discussed later. Finally it must be emphasized that the experimental difficulties involved in the accurate measurement of the absorption are great. In general the experiments were conducted so that the concentration of $SO_2$ in the air was reduced only about 5 to 15 per cent. in passing through the fumigation chamber. An error of 1 per cent. in the measurement of the difference in concentration between intake and outlet would therefore cause an error of 7 to 20 per cent. in the absorption value.

Equation 1 has been fairly well confirmed by a few carefully executed experiments conducted in 1931, which have been reported in detail elsewhere (**8**). The absorption of sulphur dioxide was determined both by gas analysis and by analysis for total sulphur of samples of leaves taken immediately before and after the fumigation. These data are plotted in chart C, figure 2. The sulphur analyses show somewhat larger absorption values than do the gas analyses, probably owing to the fact that in taking the leaf samples there was a tendency to pick an unduly large proportion of the upper leaves, which had absorbed the gas more freely than the less active lower leaves. The equation and statistics of the best fit straight line through the absorption values, determined by gas analysis alone, are as follows:

$$x = 713 + 33.9y \quad \text{.....(1a)}$$
$$n = 7$$
$$r = 0.932$$
$$S_x = 244 \text{ p.p.m.}$$
Average duration of fumigation = 1.1 hours.

The equation of the line determined by the leaf analyses alone is:

$$x = 1273 + 30.0y \quad \text{.....(1b)}$$
$$r = 0.825$$

$S_x = 389$ p.p.m.

and by the average of the gas and leaf analyses:

$$x = 995 + 31.9y \quad \text{.....(1c)}$$

$r = 0.900$

$S_x = 289$ p.p.m.

These equations represent experimental conditions comparable with those which obtained in equation (1). Equation (1c) is plotted as curve E in figure 2. As already mentioned, great care was exercised in carrying out these particular experiments, so that in spite of their meager number they may be regarded as offering definite confirmation of equation (1). The differences are within the observed experimental errors and variations.

Three series of short fumigations of about 15–20 minutes' duration have also been carried out. Because of time required to displace the large volume of air in the cabinet, the concentration of $SO_2$ in these experiments rose rapidly to a maximum over a period of 4–6 minutes, and after remaining approximately constant at this level for about 14–18 minutes, fell to zero over a period of 4–6 minutes, unless the humidity was very high, in which case the cabinet continued to lose $SO_2$ for about 20 minutes after the supply had been stopped. The average duration of the peak concentration in these experiments was 17 minutes, but appreciable amounts of $SO_2$ were present for 21 minutes on the average, and detectable amounts were registered for 28 minutes. Owing to these rapidly changing conditions in the cabinet, accurate absorption data could be obtained only if two $SO_2$ autometers were sampling continuously from the intake and outlet of the system. In practice each machine sampled from both intake and outlet, but the operation was timed so that one drew its sample from the intake while the other was sampling from the outlet. The machines were stopped momentarily in the middle of the experiment to reverse the source of sampling in order to eliminate machine differences.

The absorption values in these experiments are plotted in figure 2. The two series at high light intensity conform to the equation:

$$x = 667 + 20.2y \quad \text{.....(7)}$$

$n = 14$

$r = 0.779$

$S_x = 234$ p.p.m.

Average duration of fumigation = 0.35 hours.

The data of all three series conform to equation (8), which is shifted somewhat to the right of equation (7). This shift lends support to the statement previously made that the absorbed gas is slightly less effective in destroying leaf tissue in the shade than in direct sunlight.

$$x = 731 + 20.6y \quad \text{.....(8)}$$

$n = 20$

$r = 0.664$
$S_x = 315$ p.p.m.
Average duration of fumigation = .35 hours.

Equations (7) and (8) indicate considerably smaller absorptions for a certain amount of leaf destruction than equations (1) to (6). This means that if a definite amount of leaf destruction is produced in different time periods, the longer fumigations require lower concentrations of gas but greater total absorptions than the shorter fumigations. Further confirmation of this fact is evident in the case of long-continued fumigations at very low concentrations. For example, the analytical data of an experiment have been presented (**8**) in which alfalfa leaves absorbed, during a period of 19 days, nearly 12,000 p.p.m. sulphur, equivalent to nearly 24,000 p.p.m. sulphur dioxide calculated on the dry weight of the leaf tissue. The sulphur was largely retained in the leaves as sulphate. In this experiment only 1.0 per cent. acute markings were produced, owing to accidental peaks of concentration, although chlorotic markings had developed at the end of the experiment to the extent of 14 per cent. of the leaf area. It is suggested that the chlorotic markings were caused by this large accumulation of sulphate, which finally may have interfered with cell functions, including that of chlorophyll formation.

### General time-concentration-absorption equations for alfalfa

It seems evident from the above discussion that the amount of injury caused by a given quantity of sulphur dioxide varies with the rate of absorption of the gas; *i.e.*, a given sufficient amount of gas, absorbed in a short time period, will cause more leaf destruction than if the absorption period is more protracted. It also seems evident that some gas can be absorbed without causing any leaf destruction, the quantity thus disposed of being greater the more slowly it is absorbed. Chemical evidence in a following paper suggests that the absorbed sulphur dioxide is largely and rapidly oxidized and neutralized in the leaf. It is evident that the sulphate radical which is a normal and considerable constituent of the tissue is practically non-toxic, and if the rate of absorption does not exceed a certain threshold value, the sulphur dioxide can all be oxidized and neutralized without causing injury to the cells. The more the rate of absorption exceeds this value, the less completely will it be oxidized and neutralized and the more injury will it cause. As suggested above, in the case of sufficiently long-continued fumigations at low concentrations, it is possible that the accumulation of sulphate finally reaches a value which may interfere with the cell functions, possibly either by exceeding the buffer capacity or by producing a salt effect on the cell components. Alfalfa at Salt Lake seems to have a tolerance for sulphur dioxide absorbed thus slowly some thirty times greater than when

absorption is rapid. The fumigations on which the equations and conclusions in this paper are based were of such short duration that large accumulations of sulphate were not built up, and therefore no account is taken of this factor. The limitations of the absorption equations thus imposed will be discussed later. The data represent primarily the toxic action of sulphur dioxide itself, with such partial conversion into non-toxic forms as occurs in these short periods of fumigation.

The absorption leaf-destruction relations developed here are of limited scope, since they are concerned with fumigations of two durations only. More general relations are needed connecting leaf destruction with such variables as duration of fumigation, concentration of sulphur dioxide in the air, rate and quantity of absorption by the vegetation, and rate of conversion of the absorbed $SO_2$ to a less toxic form. A simple mathematical treatment is outlined below, by which some of these general relations may be found.

Consideration of equation (1) indicates that the absorption value x is made up of three principal variables: time, t; concentration of $SO_2$ in the air, C; and an absorption factor, A, which is a measure of the activity of the plant in absorbing the gas and varies with the external environment as well as internal conditions. The equations can therefore be written in the more general form:

$$x = x_0 + Ky \qquad (9)$$

or

$$x = tAC = tAC_0 + Ky \qquad (10)$$

where

$$x_0 = tAC_0$$

represents absorption conditions attending incipient leaf destruction ($y = 0$), and K is a constant. For definite values of y, the term Ky is constant and can be rewritten in the form, kA (k is another constant), so that

$$tAC = tAC_0 + kA \qquad (11)$$

For each value of y, the absorption is thus a linear function of t. Since equation (10) has been evaluated for fumigations of two durations, as represented by equations (1) and (7) for example, it is possible to solve equation (11) for its constants assuming definite values of y. A family of curves can thus be calculated showing the amounts of absorption in different time periods which can cause any specific degree of leaf destruction. To illustrate, assuming incipient leaf destruction, the absorption is 822 p.p.m. in 1.35 hours (equation 1) and 667 p.p.m. in 0.35 hours (equation 7). Substituting these values and solving for the constants, equation (11) becomes:

$$tAC = 610 + 155t \qquad (12)$$

Equation (12) states that a minimum absorption of 610 p.p.m. $SO_2$ (based on the dry leaf tissue) would cause incipient leaf destruction if the gas could be taken up instantaneously ($t = 0$). If the rate of absorption is less rapid, additional sulphur dioxide, proportional in amount to the time required for the whole quantity to be absorbed, is necessary to produce this effect.

If equations (5) and (8), which also represent similar external conditions, are employed, somewhat larger constants are obtained but the relations are not greatly changed:

$$tAC = 664 + 188t \quad \text{.....(12a)}$$

By similar operations, employing equations (1) and (7), 50 per cent. leaf destruction yields the equation:

$$tAC = 1350 + 925t \quad \text{.....(13)}$$

and 100 per cent. leaf destruction:

$$tAC = 2090 + 1700t \quad \text{.....(14)}$$

Equations (12), (13), and (14) are plotted in figure 3. The intercept on the Y axis, or the first constant in the equations, represents the amount of absorption that would be required to produce the specific effect if the gas could all be added instantaneously; that is, 2090 p.p.m. for 100 per cent., 1350 p.p.m. for 50 per cent., and 610 for incipient leaf destruction. These values would be increased to 3790, 2275, and 765 p.p.m. respectively, if the absorption period were one hour. These curves are not dependent on the value of A in equation (11), since this factor and C may vary reciprocally without changing the value of the absorption in any given case. The curves illustrate in striking manner the inactivation of part of the absorbed gas as the rate of absorption falls off, or conversely they show that a given amount of absorbed gas is more active the more quickly it is taken up by the plant.

If sufficiently large values of the time (t) are inserted in equations (12) to (14), quantities of absorbed $SO_2$ are indicated which are physically impossible. It is clear, therefore, that the equations must be used subject to limitations imposed by the biological system. As already pointed out, the equations were developed from data obtained in fumigations of comparatively short duration, and their extrapolation to fumigations of longer duration must be made with caution. This matter will be treated more fully in a subsequent paper. It may be pointed out that the equations will remain valid for larger values of the time the smaller the amounts of leaf destruction under consideration. Assuming the suggested interpretation of the reactions,—that part (or all) of the sulphur dioxide is converted into the non-toxic sulphate form and if sufficient gas is absorbed the remainder exerts an immediate direct harmful action,—the accumulated sulphate will probably remain non-toxic as long as: (1) it can be neutralized as rapidly

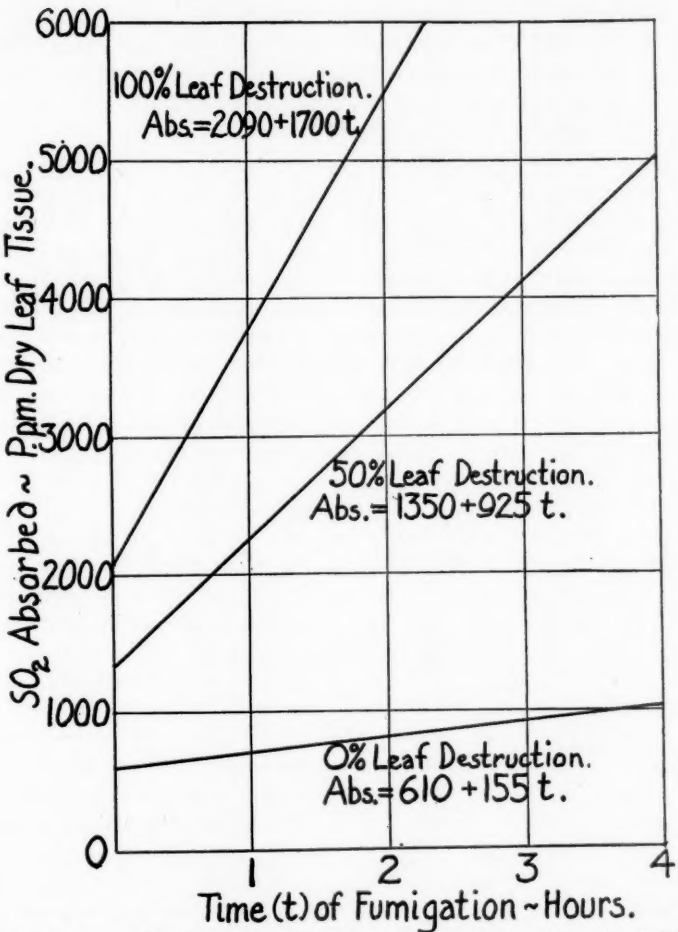


FIG. 3. Curves showing amounts of sulphur dioxide which must be absorbed in different time periods in order to produce three different stages of leaf destruction in alfalfa.

as it is formed, although it cannot be said at this time how much free sulphuric acid the cell can tolerate; and (2) its total concentration does not interfere with the cell metabolism. The first condition will depend on the quantity of bases in the system and will doubtless be different in plants grown on different types of soil. No information is available at present as to whether the second condition is subject to appreciable variation in dif-

ferent localities. It has been observed that in the presence of as much as 1.5 to 2.0 per cent. of sulphur as sulphate in the dry leaf tissue of alfalfa grown at this laboratory, the plants are normal, as indicated by yield, composition, and appearance.

By eliminating A from equation (11) we have:

$$tC = tC_0 + k \quad \ldots\ldots (15)$$

which is identical in form with an exposure equation developed by O'GARA (3, 5) and which will be discussed later. Equation (15) indicates that there is a reciprocal relation between time of exposure and concentration of the $SO_2$ in the air in producing a definite amount of leaf destruction. This relation will hold, however, only if the rate of absorption A is constant in the whole series of exposures embraced by equation (15). If A is not constant in the whole series, equation (15) does not follow from equation (11).

The factor A is a measure of the absorptive rate of unit (dry) weight of the leaves and is mathematically independent of the duration and intensity of the fumigation. Physiological and environmental influences will cause the value of A to vary over a wide range and the absorbed $SO_2$ may cause it to change during a short fumigation treatment. Examination of the 1928 to 1931 absorption data reveals that the value of A ranged from a minimum of about 30 to a maximum of 650 in the different daylight experiments. The dimensions of A were so chosen that when the time was expressed in hours and the concentration of $SO_2$ in the air in parts per million by volume, the absorption was given as parts per million of the dry leaf tissue. The same maximum value of A (650) was observed in both the 15–20-minute and the 60–100-minute fumigations, but A was generally smaller in the longer treatments under comparable conditions owing to the fact that it frequently decreased during the course of the treatment.

If it is desired to know the time-concentration exposure relations which will produce a given amount of leaf destruction with a definite rate of absorption, it is necessary only to divide the appropriate equation of the family to which equations (12) to (14) belong by the chosen absorption rate factor A. For example, if the equation representing incipient marking under conditions of maximum sensitivity is sought, equation (12) should be divided by:

$$A = 650$$

$$tC = 0.94 + 0.24t \quad \ldots\ldots (16)$$

A corresponding equation for 50 per cent. leaf destruction is:

$$tC = 2.1 + 1.4t \quad \ldots\ldots (17)$$

and for 100 per cent. leaf destruction:

$$tC = 3.2 + 2.6t \quad \ldots\ldots (18)$$

These curves are plotted in figure 4, and represent minimum values of the

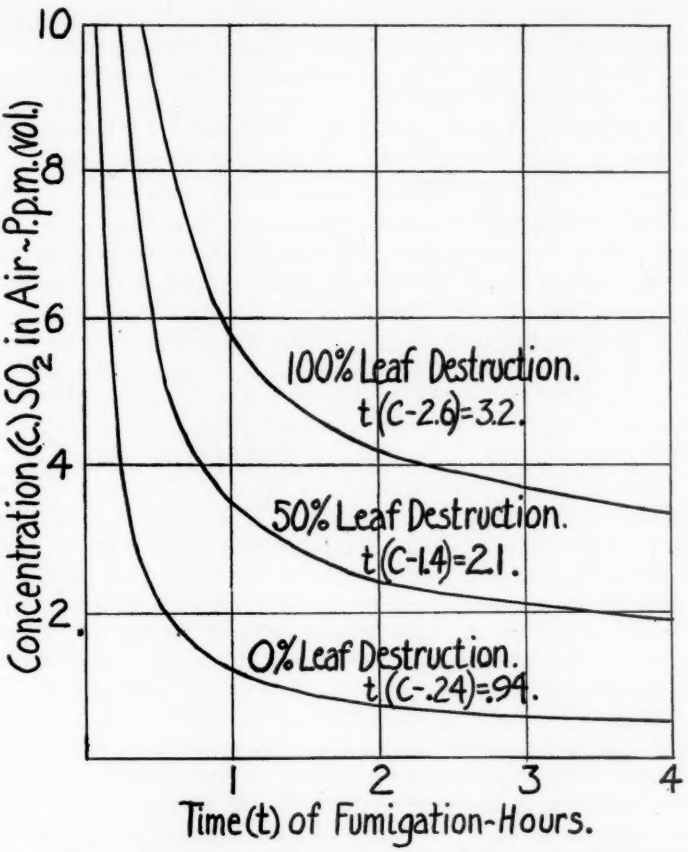


FIG. 4. Curves showing concentrations of sulphur dioxide which will produce three different stages of alfalfa leaf destruction in different time periods under conditions of maximum absorption (A = 650).

sulphur dioxide concentration in the air which will produce a trace, 50, or 100 per cent. leaf destruction respectively in alfalfa, under conditions of most rapid absorption. Actual fumigations will generally require higher concentrations than those indicated, because an absorption rate of A = 650 is not often attained, particularly in the longer fumigations which usually show a decreasing value of A as the fumigation proceeds.

As already mentioned, O'GARA developed an exposure equation like equation (15). He observed the concentrations of the gas which produced incipient markings in alfalfa with different times of fumigation. Then

using an empirical equation connecting gas concentrations with relative humidity, he adjusted his concentration values to correspond with 100 per cent. relative humidity, which was assumed to represent a condition of maximum sensitivity of the alfalfa. These adjusted values are plotted in figure 5 against the time of fumigation. The curve O'GARA drew through the *minimum* values of concentration conforms to the equation:

$$tC = 0.92 + 0.33t \quad \text{.....(19)}$$

This curve is clearly a reasonable interpretation of the data, although slightly different constants might have been chosen. The fact that it is not materially different from equation (16) lends support to the mathematical treatment of the absorption data presented.

O'GARA'S interpretation of equation (19) was as follows:

"With all environmental factors remaining the same, the active part of the gas necessary to produce a certain effect upon the plant cell, multiplied by the time through which it acts is constant" (**3, 5**).

This is more clearly shown if the equation is rearranged:

$$t(C - 0.33) = 0.92 \quad \text{.....(19a)}$$

According to O'GARA, the constant 0.33 represents a threshold concentration to which the plants can be subjected indefinitely without producing markings under conditions of maximum sensitivity, and the "active" part of the gas in any fumigation can be found by subtracting 0.33 p.p.m. from the actual concentration. This interpretation of the exposure factors is analogous to that just given for the absorption values. The similarity of the concepts is based on the implicit condition that throughout a given series of fumigations a definite fraction of the gas is absorbed. In that case absorption is directly proportional to exposure concentration. Ordinarily this assumption is not justified and a large part of the fumigation data will not conform closely to these simple relations, as indicated by many of the individual points in figure 5. Of course both the exposure and the absorption data are subject to variations such as are represented by the dispersion of the points in figures 1 and 2.

Further confirmation of the preceding mathematical treatment and interpretations may be found in the barley fumigation data of the Selby Smelter Commission (**2**). Two examples which are typical of the results may be quoted: Severe lesions were produced by 8–10 p.p.m. $SO_2$ in one-half hour; 5 p.p.m. in one hour; or by 1 p.p.m. for 43 hours intermittently on 6 days. Slight leaf destruction was produced by 6, 12, 18, or 27–36 fumigations, each of 10 minutes' duration, when the gas concentration was 5, 3, 2, or 1 p.p.m. respectively. While no mathematical treatment of the Selby data has been attempted, it is clear that equations of the type of (15) could be worked out for at least several of the series of fumigation treat-

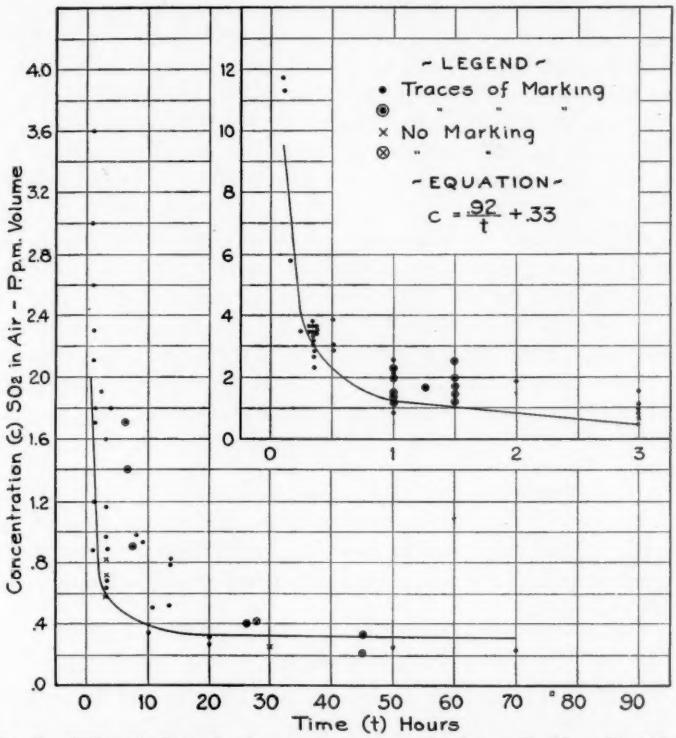


FIG. 5. O'GARA'S data showing concentrations of sulphur dioxide, adjusted to 100 per cent. relative humidity by an empirical equation involving the relative humidity of the air, which cause no more than traces of markings on alfalfa in different times of exposure. Circled points represent data obtained by the writers.

ments. Again, such regularities as might be found would depend on a duplication of conditions in a series so that the absorption factor would be approximately constant.

### Toxic dosage of $SO_2$ for the mesophyll cells

It seems desirable to attempt to estimate, on the basis of the preceding data, the amount of $SO_2$ which is lethal to the absorbing cells of the leaf. It has been shown that the absorption in an infinitesimally short time period of 610 p.p.m. $SO_2$, calculated on the dry leaf tissue, produces incipient marking, and that the absorption of 2090 p.p.m. under similar conditions causes complete leaf destruction. The different cells of a leaf do not absorb the gas with equal rapidity, because of unequal areas exposed to the gas.

Accordingly the first cells to show injury have undoubtedly absorbed considerably more than the average amount per cell found for the whole leaf. On the other hand, absorption does not stop when injury appears, and therefore the absorption which attends complete leaf destruction probably represents much more than the lethal dosage for a considerable portion of the leaf. The most probable value of the lethal dosage for the injured section lies between these extremes, and is represented by the average, 1350 p.p.m., dry basis, or about 270 p.p.m. $SO_2$ based on the green weight of the leaf. It should be noted that the absorption occurs principally in the mesophyll, since this tissue is particularly adapted by its very large and continuously moist exposed area for the rapid absorption of gases. On the other hand, the water proofed epidermis and the compact vascular tissue, with its comparatively small area exposed to the air, probably play an insignificant part in this gaseous absorption. If these latter tissues represent 50 per cent. of the leaf substance, which seems to approximate the correct value, the toxic dosage for the remaining mesophyll would be twice the average for the whole leaf. The figures, therefore, of

2700 p.p.m. $SO_2$ in dry mesophyll cells

and

540 p.p.m. $SO_2$ in green mesophyll cells

are suggested as probably representing the order of magnitude of the lethal quantity of this gas.

## Summary

1. The amount of sulphur dioxide absorbed by the leaf tissue of alfalfa has been measured in a considerable number of fumigation treatments.

2. As a first approximation, the extent of leaf destruction is a linear function of the amount of $SO_2$ absorbed in a given time period.

3. An appreciable amount of gas can be absorbed without causing any leaf destruction.

4. The amount of leaf destruction attending a given amount of absorption increases as the rate of absorption is increased.

5. For a definite amount of leaf destruction, for relatively high exposure, the amount of absorption is a linear function of the time required for the absorption.

6. An absorption factor, A, which depends on the activity of the leaf in taking up the gas, is discussed. It is shown that if this factor is constant in a series of experiments, the absorption equation can readily be transformed into time-concentration exposure equations. In this way the fumigation data of O'Gara and the absorption data in this paper lead to practically identical equations for the exposure conditions which produce incipient marking in alfalfa under conditions of maximum sensitivity. Fur-

ther, it is possible to calculate from the absorption equations the exposure condition for any specified amount of leaf destruction or value of A.

7. The evidence indicates that when the rate of absorption exceeds a certain threshold value, a specified amount of injury appears in a time which is proportional to the difference between the actual rate of absorption and the threshold rate. The cells of the leaf can dispose of a certain amount of the gas by oxidation and neutralization, but injury occurs if the gas is supplied so rapidly that these processes cannot be maintained, thus permitting a sufficient accumulation of unoxidized or unneutralized suphur dioxide.

8. Assuming that the lethal dosage of sulphur dioxide is added to the leaf instantaneously, and that it is all absorbed by the mesophyll cells, which represent about one half of the leaf substance, it is suggested that the limit of tolerance to the gas of these cells is approximately:

2700 p.p.m. in dry tissue

and

540 p.p.m. in fresh tissue.

DEPARTMENT OF AGRICULTURAL RESEARCH
AMERICAN SMELTING & REFINING COMPANY
SALT LAKE CITY, UTAH

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# INFLUENCE OF DIFFERENT QUANTITIES OF MOISTURE IN A HEAVY SOIL ON RATE OF GROWTH OF PEARS[1]

M. R. Lewis, R. A. Work, and W. W. Aldrich

(WITH FIVE FIGURES)

## Introduction

In 1930 studies were initiated in the Medford area to determine so far as possible the best methods of irrigation of the heavy soils of the area for the production of large yields of high quality pears. One of the primary problems encountered was that of the effect of different soil moisture conditions at different times during the growing season on the yield of fruit. During the first two years complete records of soil moisture conditions and of the size and yield of fruit at harvest were secured. Study of these results brought out the urgent need for more detailed data on the effect of moisture conditions on the rate of growth of fruit from day to day. This information was secured during the 1932 growing season. An analysis of the rate of growth of fruit throughout the season as compared with the corresponding soil moisture conditions is presented in this report. The results obtained from this analysis are applied to the soil moisture conditions of the two earlier years with a satisfactory check on the size of fruit at harvest.

During each of the three years, studies were conducted on two commercial orchards designated as the Fitch and Klamath orchards. During 1932 additional work was done at the Medford Experiment Station. Both soils and trees in these orchards are extremely variable, but in each case the plots were selected for uniformity of soil type and tree growth. Mature bearing trees were selected. The treatment of the different plots in each orchard up to the time of the beginning of these experiments was uniform so far as is known. The trees on the Fitch orchard are Bartletts while on both the Klamath and Medford Experiment Station orchards the variety is Anjou, all on French roots.

[1] The data reported in this paper have been secured in the course of studies on the irrigation of pears carried on cooperatively by the U. S. Department of Agriculture and the Oregon Agricultural Experiment Station near Medford, Oregon. All of the work in 1930 and 1931 and the irrigation and soil moisture studies in 1932 were conducted by R. A. Work under the direct supervision of M. R. Lewis. Detailed studies of fruit growth and tree response were conducted in 1932 by W. W. Aldrich. The method of interpreting the data as reported in the present paper has been developed by the senior writer.

Presented for publication as technical paper no. 207 with the approval of the Director as a contribution from the Medford Branch Experiment Station, Oregon Agricultural Experiment Station.

The climate is semi-arid with a comparatively long growing season and almost continuous sunshine during the summer months.

Irrigation was by the furrow method, six to eight furrows being used in each middle. Irrigation water was available on demand except after early July in the 1931 season. The Fitch and Klamath orchards were cultivated once after each irrigation. The Medford Experiment Station orchard was cultivated once in early summer when the winter cover crop was disced in, and again once late in the summer.

The soils in these orchards are very heavy, locally known as "sticky," and are typical of a large part of the pear orchard land in the Medford area. The soil of the Fitch orchard is mapped as Meyer silty clay loam and that of the other two orchards as Meyer clay adobe. Analyses of typical samples of these soils are reported by the Bureau of Soils (**7**). A sample of the Meyer clay adobe from an adjoining field of the Medford Experiment Station had the following mechanical analysis (**6**): total sand 36.4 per cent., silt 25.3 per cent., and colloidal clay 38.1 per cent.

The soil is from 3 to 6 feet deep and rests on a somewhat disintegrated shale. The water table is below the surface of the rock at all times except occasionally just after a heavy irrigation when it may rise above the rock surface for a few days.

## Plan of experiments

In each orchard four plots were laid out with border rows and with from 5 to 16 trees in inner rows which were considered satisfactorily uniform within each orchard for use in the study of the effect of soil moisture conditions on the growth and yield of fruit. The moisture content of the soil in the different plots was permitted to drop to various percentages of the available capacity of the soil before being replenished by irrigation.

Samples for soil moisture determinations were taken just before and as soon after each irrigation as it was possible to do so without too much difficulty with the sticky mud. Samples were also taken at approximately 2-week intervals when periods between irrigations permitted. Samples were taken in increments of 1 foot in depth, starting below the mulch and extending to the bed rock. Representative samples of the soil of each plot secured in the Fitch and Klamath orchards from five holes and in the Medford Experiment Station orchard from twenty holes were used for all determinations. Methods used in determining soil moisture constants in these studies are described elsewhere (**9**).

The location of the feeding roots of the trees in these orchards has been investigated both by actual count and weight of roots recovered from trenches and by study of the rate of extraction of moisture from the soil. The data (**2**) indicate that almost all of the roots are in the upper 4 feet,

and that of those about 89 per cent. are in the upper 3 feet. The rate of loss of soil moisture from depths below 3 feet is very slow and as a result the variation in soil moisture below that depth is comparatively small. For these reasons the moisture content of the upper 3 feet of the soil has been used as representing the moisture available to the trees. The moisture content is presented in terms of the percentage of the available capacity present at any time. In other words, the moisture content of the plots shown on the curves as 0 per cent. is the wilting point and the content shown as 100 per cent. is the field capacity.

The rate of growth of fruit during 1932 in the Fitch and Klamath orchards was determined by measuring the circumferences of 45 pears on each plot at intervals of from 3 to 7 days. These measurements were made from May 17 to August 11, about one week before the first picking of the Bartlett pears on the Fitch orchard, and to September 7, two days before picking of the Anjous on the Klamath orchard. At the Medford Experiment Station orchard 90 fruits per plot were measured at 3-day intervals from May 23 to September 8.

The actual volume of individual fruits of these varieties approximates that of a sphere having the same circumference. The volumes of spheres corresponding to measured circumferences rather than actual volumes of fruits have therefore been used throughout this study.

The fruit from each plot was run through mechanical graders in the packing houses separately and the number of pounds of each size of fruit was determined.

## Results

Table I gives the average field capacity, wilting point, and available capacity of the soil in the three orchards. Results for individual plots are reported elsewhere (**9**).

Study of the effect of different soil moisture conditions on the yield of fruit (**8**) indicated that in these plots the trees suffered from lack of soil moisture before the soil in the upper 3 feet had reached the wilting point. ALDRICH and WORK (**1**) have shown that differences in the moisture content of the upper 3 feet of the soil below 50 per cent. of the available capacity but well above the wilting point resulted in differences in fruit size and branch growth, but that above 60 per cent. of the available capacity the growth of fruit was not materially decreased. The points plotted on figure 1 represent the rate of growth of fruit on all plots and for all periods during which the soil moisture was above 60 per cent. of the available capacity. These curves may be considered as representing the rate of growth during the year 1932 when water was not a limiting factor, or, so far as this study is concerned, as the "normal" rate of growth, and have been so designated.

TABLE I

SOIL MOISTURE CONSTANTS FOR VARIOUS DEPTHS IN THE FITCH, KLAMATH, AND MEDFORD EXPERIMENT STATION ORCHARDS

| ORCHARD | 0–1 FEET | | | 1–2 FEET | | | 2–3 FEET | | | 0–3 FEET | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | FIELD CAPACITY | WILTING POINT | AVAILABLE CAPACITY | FIELD CAPACITY | WILTING POINT | AVAILABLE CAPACITY | FIELD CAPACITY | WILTING POINT | AVAILABLE CAPACITY | FIELD CAPACITY | WILTING POINT | AVAILABLE CAPACITY |
| | % | % | % | % | % | % | % | % | % | % | % | % |
| Fitch | 24.7 | 13.3 | 11.4 | 27.4 | 15.7 | 11.7 | 28.5 | 17.4 | 11.1 | 26.9 | 15.5 | 11.4 |
| Klamath | 28.7 | 15.8 | 12.9 | 28.0 | 17.2 | 10.8 | 27.3 | 16.7 | 10.6 | 28.0 | 16.6 | 11.4 |
| Medford Exp. Sta. | 34.5 | 17.2 | 17.3 | 32.0 | 17.5 | 14.5 | 30.3 | 17.6 | 12.7 | 32.3 | 17.4 | 14.9 |

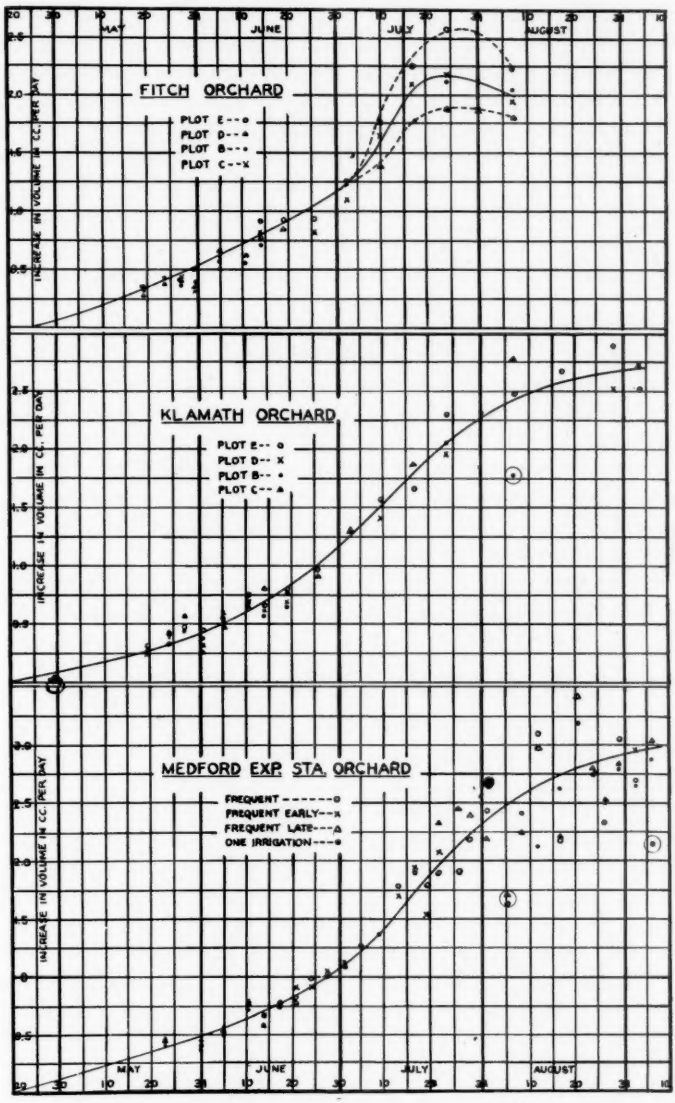


FIG. 1. Rate of growth of pears in cc. per day during 1932 in the Fitch, Klamath, and Medford Experiment Station orchards (the so-called "normal" rate of growth).

It is obvious that this curve will be affected by many factors other than the soil moisture conditions. The variety of fruit, climatic conditions, age and vigor of the trees, number of pears carried by the trees in comparison with their leaf area, and, perhaps, the size of trees, both above and below ground, have a bearing on the shape of the so-called "normal" growth curve. For these reasons separate curves have been plotted for each of the three orchards. In each orchard all treatments (including pruning, thinning, cultivating, etc.) except irrigation were as nearly alike as possible for all four plots. Within each orchard and for the one season it is believed that all conditions except soil moisture and, in one or two cases especially mentioned later, number of fruits per tree, are the same. It will be noted that on all three curves there are certain periods when the measured rate of growth is markedly slower or faster than is indicated by the smooth curves. Probably the most marked instance is for the period centering on May 31 or June 1. Many of these variations seem to be due to atmospheric conditions. In order to avoid introducing an error into the subsequent computations, the normal rate of growth for each period for the year 1932 is taken as the arithmetic mean of the rate for all the plots in which the moisture content was over 60 per cent. of the available capacity during that period, rather than the rate shown by the smooth curve.

It will be noted that the rate of growth curve for the Fitch orchard is broken into three parts late in the season. Study of the conditions on the plots in this orchard leads to the belief that the differences shown by plots E and D from the more central position of the plotted points for the other plots are due to differences in the leaf-fruit ratio, or the number of fruits borne by the trees in the various plots. Plot D apparently had a lower leaf-fruit ratio than plot E. The curve shown as a solid line is assumed to represent normal growth rate in the following discussion.

The curves of figure 2 show the deviation from the normal rate of growth of fruit for each orchard plotted against the soil moisture content. The difference between the measured rate of growth for each period for each plot and the normal rate of growth as just defined has been expressed as a percentage of the normal rate. This percentage deviation has then been plotted against the corresponding average available moisture content of the upper 3 feet of soil expressed as a percentage of the available capacity. These curves show a very definite relation between the rate of growth of the fruit of pear trees in this heavy soil and the amount of soil moisture in the root zone of the trees.

All three of these curves cross the line of normal growth at a moisture content of about 75 per cent. of the available capacity of the soil, and the maximum rate of growth is not attained below 80 or 85 per cent. The curves are very similar in shape and indicate that the effect of a shortage of

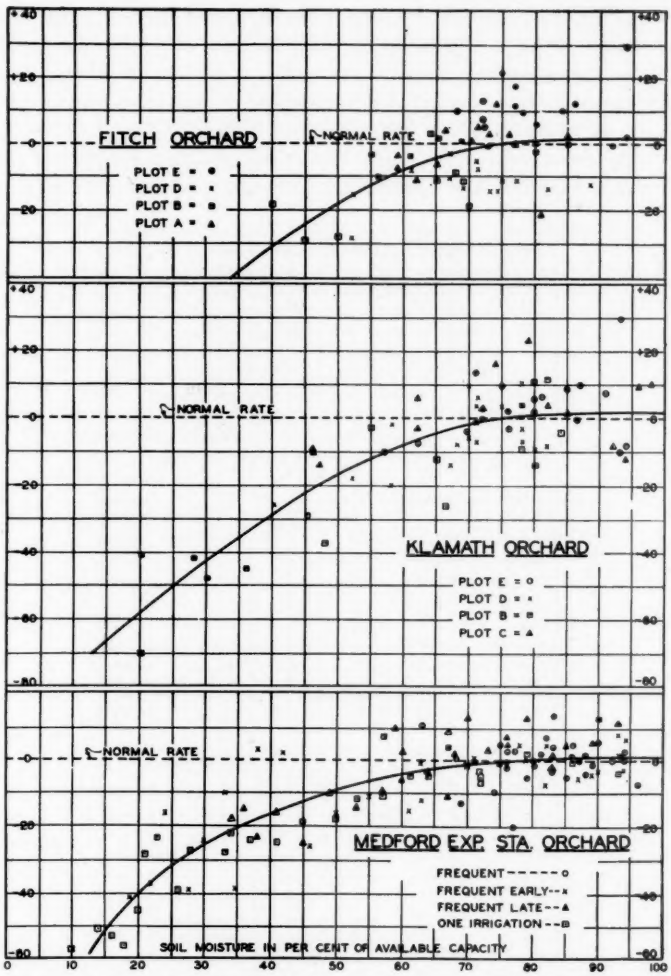


FIG. 2. Deviation from "normal" rate of growth as compared with percentage of available moisture present in the upper 3 feet of soil in the Fitch, Klamath, and Medford Experiment Station orchards in 1932.

soil moisture is similar in the three orchards with the two varieties of pears. When the soil moisture is reduced to 20–35 per cent. of the available capacity of the upper 3 feet, the rate of growth of fruit is 40 per cent. less than the normal rate of growth. This indicates that the lower limit of the range of soil moisture producing most rapid growth of pears in this soil is much higher than the wilting point.

These curves indicate that under the conditions of these experiments soil moisture is an important limiting factor when the moisture content of the upper 3 feet is less than about 75 per cent. of its available capacity. At higher moisture contents other factors appear to become limiting to such an extent as to overshadow the effect of soil moisture. Other factors undoubtedly have some effect under lower soil moisture conditions.

Figures 3, 4, and 5 show the moisture conditions in the plots during the

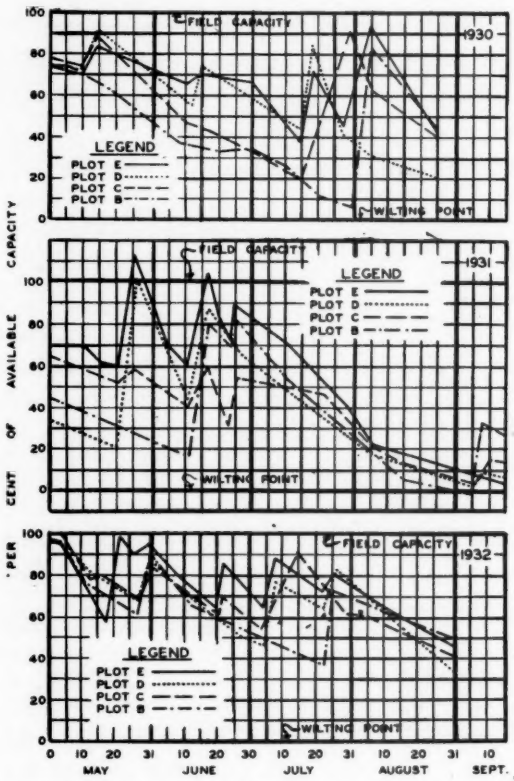


FIG. 3. Average moisture content of upper 3 feet of soil in the different plots of the Fitch orchard in 1930, 1931, and 1932, expressed as percentage of available capacity.

three years. The moisture content shown is the average for the upper 3 feet and is presented as the percentage of the available capacity present. Examination of original data shows that after an irrigation, the soil moisture drops more rapidly in the first foot than in either of the deeper feet, and that, in general, the second foot loses water more rapidly than the third

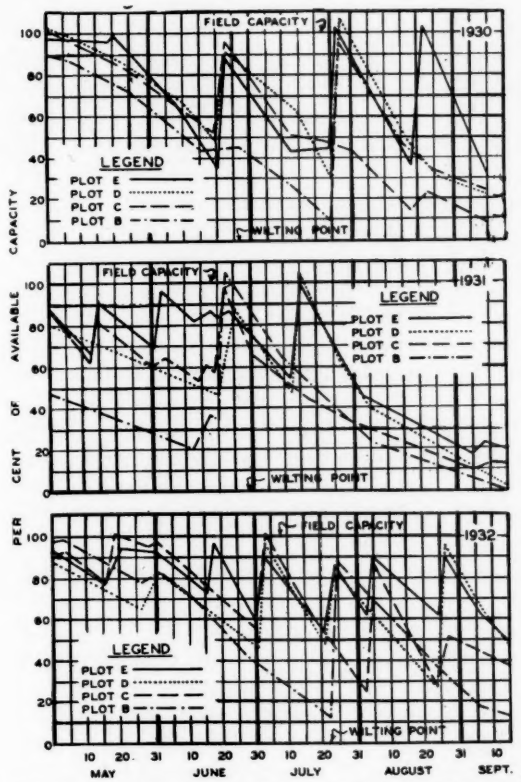


FIG. 4. Average moisture content of upper 3 feet of soil in the different plots of the Klamath orchard in 1930, 1931, and 1932, expressed as percentage of available capacity.

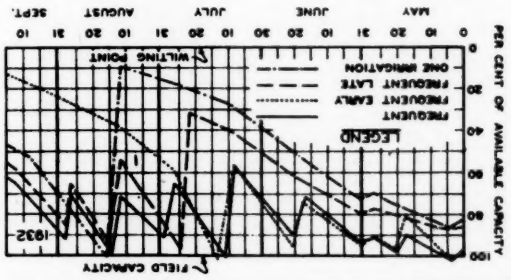


FIG. 5. Average moisture content of upper 3 feet of soil in the different plots of the Medford Experiment Station orchard in 1932, expressed as percentage of available capacity.

foot. In spite of this, in no case during the season of 1932 did the moisture content of the first foot of soil reach the wilting point.

In order to arrive at a check on the conclusion drawn here that the rate of growth of pear fruits is closely correlated with the soil moisture content of the upper 3 feet of soil, an estimate of the average size of the fruits harvested from these plots during all three years has been prepared. This method has been necessary because no measurements of the growth of the fruits were made during the growing seasons of 1930 and 1931.

Knowing the number of pounds of fruit to a packed box and also the number of fruits of each size to each packed box, it is possible to compute the number of pears of each size yielded by each plot. From data furnished by officials of local packing houses the volume of individual pears of the various sizes was determined. The average size of the individual fruits for each plot was then determined in the following manner. For each plot the total volume of fruit of each size was determined by multiplying the number of fruits by the volume of each fruit. The sum of the total volumes of fruits of each size then gave the total volume for the plot, and this figure divided by the total number of fruits of all sizes gave the average size of individual fruits for the plot. It is believed that this method gives an accurate estimate of the average size.

The data of figures 3, 4, and 5 on soil moisture and of figures 1 and 2 on the normal rate of growth and the effect of soil moisture on the rate of growth of the fruit make possible an estimate of the size of the average fruit at any time during the season. This has been done for each plot and for each season of our experiments. The moisture content in the upper 3 feet of soil in each plot for each day was determined from figures 3, 4, and 5. From the curves of figure 2 the deviation from the normal rate corresponding to the soil moisture content present each day was taken. This deviation was then applied to the normal rate for the day as shown on figure 1 and the estimated growth for the day determined. By summing up the growth for all the days the size of the fruit at picking time was estimated. Table II is a summary of the results obtained by the three methods: (1) measurement of the circumference of a great number of fruits in the field; (2) computation from the packing house records of yield and size of the fruit harvested from each plot; and (3) estimation from the soil moisture records and the curves of normal growth and deviation from normal growth as influenced by variation in soil moisture.

It is felt that the very close agreement between the size as estimated from the soil moisture data and that as determined directly from the fruit, either by direct measurement or by computation from yield records for the year 1932, is remarkable. The volumes computed from yield data in the Fitch orchard are not directly comparable with the volumes secured by the

## TABLE II

SIZE OF PEAR FRUITS ON ALL PLOTS AND ALL SEASONS: AS MEASURED (COLUMN 1); AS COMPUTED FROM YIELD RECORDS (COLUMN 2); AND AS ESTIMATED FROM SOIL MOISTURE DATA (COLUMN 3)

| YEAR | PLOT E | | | PLOT D | | | PLOT C | | | PLOT B | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* |
| | *Fitch orchard* | | | | | | | | | | | |
| 1930 | ..... | 107.1* | 105.1 | ..... | 101.5* | 91.1 | ..... | 104.0* | 88.1 | ..... | 98.3* | 63.0 |
| 1931 | ..... | 144.7* | 88.4 | ..... | 131.5* | 72.3 | ..... | 127.0* | 74.9 | ..... | 125.8* | 69.9 |
| 1932 | 120.4 | 141.8* | 111.3 | 93.6 | 125.8* | 104.6 | 105.0 | 131.0* | 105.6 | 97.2 | 134.7* | 97.8 |
| | *Klamath orchard* | | | | | | | | | | | |
| 1930 | ..... | 148.0 | 170.0 | ..... | 137.2 | 155.2 | ..... | 114.0 | 120.6 | ..... | 120.0 | 133.6 |
| 1931 | ..... | 130.0 | 130.3 | ..... | 132.0 | 121.7 | ..... | 98.8 | 104.4 | ..... | 96.0 | 95.0 |
| 1932 | 184.3 | 175.0 | 178.3 | 161.0 | 162.0 | 164.7 | 181.9 | 169.0 | 155.3 | 121.5 | 122.0 | 133.4 |
| | *Medford Experiment Station orchard* | | | | | | | | | | | |
| | PLOT FREQUENT | | | PLOT FREQUENT EARLY | | | PLOT FREQUENT LATE | | | PLOT ONE IRRIGATION | | |
| 1932 | 180.4 | 180.0 | 186.3 | 157.6 | 158.5 | 157.7 | 180.6 | 180.0 | 179.2 | 143.2 | 149.5 | 146.2 |

* In the Fitch orchard the field measurements of fruit and the estimates from soil moisture conditions were made only up to the time of the first picking. As a part of the fruit was left on the trees and grew rapidly before the second picking, the size of fruits computed from the yield and size data is considerably larger than that shown by the other methods.

other two methods. The greatest discrepancy in comparable data is in the case of plot C in the Klamath orchard. A probable explanation for the marked difference between the measured volume and the volume computed from yield data in this plot is that growth measurements were made on the lower branches where the leaf-fruit ratio was larger than on the upper branches where the bulk of the crop was carried. The fact that both of these volumes are large as compared with the volume estimated from soil moisture is explained by the small number of fruits carried by the trees in this plot. It seems to be generally true that, all other things being equal, a tree with a small number of fruits will produce fruits of a larger size than will the same tree with a large number of fruits. In this case the measured volume is 17 per cent. larger and the computed volume is 8.8 per cent. larger than the estimated volume. In no other case in 1932 is the estimated volume more than 10.5 per cent. different from the comparable volume determined by other methods.

Since it was necessary to apply the so-called normal rate of growth found in 1932 to the moisture conditions found in the plots in 1930 and 1931, it is not to be expected that as close an agreement will be found in these years. Again the figures for the Fitch orchard are not directly comparable. Except for the reversal of plots C and D, which reversal is only a matter of 3 or 4 cc., the order of the sizes in the plots is the same by computation from yield data and estimation from soil moisture. The much larger sizes of fruit determined from yield data as compared with the expected sizes computed from moisture conditions in the Fitch orchard in 1931 are due to the fact that the fruit in this orchard was heavily thinned that season in anticipation of a water shortage. In the Klamath orchard the maximum difference between the two methods is 13 per cent. These data furnish convincing proof that the effect of soil moisture deficiency is reasonably well defined by the curves of figure 2.

## Discussion

As pointed out by ALDRICH and WORK (**1**), such results on heavy soils seem to lead to conclusions contrary to those arrived at by some other workers using lighter soils. HENDRICKSON and VEIHMEYER (**4**) conclude that "trees either *have* readily available moisture or *have not.*" BECKETT, BLANEY, and TAYLOR (**3**), working with citrus and avocado trees, reach the somewhat similar conclusion that "moisture is as readily available when the moisture content is one-third or two-thirds of the way between field capacity and the wilting point as it is in the thoroughly moistened soil after irrigation."

Three reasons either singly or in combination may account for the slowing down of the growth of fruit in our experiments as the quantity of water held in the root zone decreases.

(1) Perhaps soil moisture becomes actually less available to the roots of the trees as the thickness of the moisture film decreases. If this is the explanation it must become effective at film thicknesses nearly as great as those present when the moisture is held in approximate equilibrium between gravitational and capillary forces and become progressively more marked as the moisture content approaches the wilting point. The data reported by SEKERA (5) seem to support this explanation.

(2) The growing root tips or root hairs which are actively extending into moist soil may not be able to advance rapidly enough to develop sufficient water to meet the needs of the trees or they may not be able to penetrate all parts of the soil mass. When, just after an irrigation, the moisture content of the soil of the root zone is at or near the field capacity, each growing root may be supposed to be in contact with a film of available water. As the water in contact with the active root tips or hairs is exhausted, it is at first relatively easy for most of the growing points to advance and thus secure more water. It is conceivable, however, that more and more of the growing points may for one reason or another find it impossible to follow the retreating water films, and thus cease to function. It seems reasonable to suppose that this condition would be much more apt to occur in very dense and fine-textured soils such as these. As a portion of the growing roots become inactive, those remaining would become progressively less able to supply the quantity of water required to keep the fruit growing at the maximum rate.

(3) It is inconceivable that the roots of these trees can come in direct contact with every soil particle and its enveloping film of water. If this be true, there must be some movement of water from those portions of the soil not in direct contact with active root tips or hairs toward these roots before the moisture content of the soil mass can be reduced to the wilting point. The distance through which this movement will take place will depend on the completeness with which the roots are able to occupy the soil. In heavy soils such as these, the soil moisture gradient toward the active roots must be rather steep if appreciable movement is to take place. The moisture supply to the tree may then be fixed, not by the rate at which roots can take up water but by the rate at which the water can move through the soil to the roots.

It will be evident that under either of the two latter conditions the moisture content of the soil immediately in contact with active roots may be reduced to the wilting point, while the soil at some distance (perhaps a very small one) may have a much higher content of moisture. Now the moisture content of a soil sample taken by ordinary methods would be a sort of average of that held by the soil adjacent to the root hairs and that held at some distance from any active root. The moisture content disclosed by

sampling and the actual *average* moisture content of a portion or all of the root zone might then be well above the wilting point, while the actual moisture content of the soil adjacent to a portion (or in extreme cases, all) of the active roots might be down to the wilting point.

It is the opinion of the writers that some combination of the slow movement of water through this tight soil and the relatively sparse root population in the soil accounts for the effect on the growth of fruit of comparatively small changes in the average moisture content of the soil in the root zone.

## Summary

1. The field capacity of the soil in the three orchards included in the test varied from 24.7 to 34.5 per cent., the wilting point from 13.3 to 17.6 per cent., and the available capacity from 10.6 to 17.3 per cent.

2. The rate of growth during those periods and on those plots when and where the soil moisture was more than 60 per cent. of the available capacity is designated the normal rate of growth and is shown graphically.

3. The deviation of the rate of growth from this normal, expressed as a percentage of the normal, is plotted against the soil moisture in the upper 3 feet.

4. The curve resulting from this plotting shows a very close correlation between moisture content and rate of growth of fruit.

5. The sizes of fruit as measured in the field, as computed from yield records, and as estimated from soil moisture data check remarkably well.

6. It is believed that the data furnish definite proof that the rate of growth of pear fruits is markedly affected by comparatively small variations in the moisture content of the soil of the root zone, even when the moisture content is well above the wilting point.

7. The data indicate that the growth of the fruit of pear trees on this heavy soil is reduced whenever the soil moisture in the major portion of the root zone is reduced below 70 per cent. of the available capacity.

OREGON AGRICULTURAL EXPERIMENT STATION
CORVALLIS, OREGON

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# COLORIMETRIC DETERMINATION OF CAROTENE IN PLANT TISSUE[1]

WALTER C. RUSSELL, M. W. TAYLOR, AND D. F. CHICHESTER

(WITH TWO FIGURES)

## Introduction

The discovery by BORODIN (**2**) in 1883 that the carotenoid pigments could be separated into alcohol soluble and petroleum ether soluble groups has been the basis for all the procedures that have been described for the determination of carotene and other carotenoid pigments. ARNAUD (**1**), in 1887, extracted dry plant tissue with petroleum ether and used a colorimetric method for the estimation of the amount of pigment present, a carotene solution being employed as a standard. No attempt was made to separate carotene from other yellow pigments and the purity of the carotene standard was not given. In 1913, MONTEVERDE and LUBIMENKO (**9**) reported a spectro-colorimetric method for the estimation of the pigments of green leaves, and in the same year WILLSTÄTTER and STOLL (**17**) presented a method for the determination of carotene and xanthophyll which has served as the starting point for all subsequent modifications. The latter method consists essentially of acetone extraction of plant tissue, saponification of chlorophyll, separation of the carotenoids by means of petroleum ether and aqueous methyl alcohol, and the colorimetric estimation of the pigments. A petroleum ether solution of carotene or an aqueous solution of potassium dichromate served as a colorimetric standard. COWARD (**3**), in 1926, modified the procedure by making the first step the decomposition of chlorophyll, which was followed by extraction with petroleum ether and the separation of carotene from xanthophyll by aqueous methyl alcohol. The use of diethyl ether in addition to acetone in the extraction of plant tissue was introduced by SCHERTZ (**12**) in 1928. In 1923 he described a method (**13**) for the spectrophotometric estimation of carotene. SPRAGUE and SHIVE (**15**), 1929, employed the method as modified by SCHERTZ (**12**), except that they used petroleum ether rather than diethyl ether as a solvent for the carotenoids. These investigators (**15**) and SPRAGUE and TROXLER (**16**) developed a color standard of dye solutions for use in colorimetric readings. Pyridine has been employed by SMITH and SMITH (**14**) for the extraction of small quantities of fresh fruit, the pigments being transferred to petroleum ether. Recently, KUHN and BROCKMANN (**6**) have described the use of petroleum ether and methyl alcohol in the extraction of plant tissue and the subsequent separation into

[1] Journal Series paper of New Jersey Agricultural Experiment Station, Department of Agricultural Biochemistry.

petroleum ether and aqueous methyl alcohol phases. After these steps, saponification with alkali in the petroleum ether phase is followed by a further partition between petroleum ether and 90 per cent. methanol. The use of suitable absorption agents allows the separation of $\alpha$ and $\beta$ carotene from lycopin in the petroleum ether phase. A solution of azobenzene was used as the colorimetric standard.

The original objective of this investigation was the correlation of the carotene content of plant tissue with vitamin A potency, determined biologically, and with the biological response when isolated carotene was fed. Hence, an accurate method for the determination of carotene which would give consistently reproducible results was necessary. In our hands the method as modified by SPRAGUE and SHIVE (**15**) when used for a series of determinations on the same sample of dried green plant tissue did not give results which agreed within the experimental error allowable in the work projected; therefore a critical study was made of the original WILLSTÄTTER and STOLL (**17**) procedure as modified by SPRAGUE and SHIVE.

## Analytical procedure

### PREPARATION OF SAMPLE

As the first step in the analytical procedure it was necessary to ball-mill the dried plant tissue until a fine powder was obtained. It has been our practice with artificially dried alfalfa to reduce the chopped product to a coarsely divided state by means of a Wiley mill and then to subject it to ball-milling.

In the case of fresh plant tissue (alfalfa), the whole plant was cut into 5–10 mm. pieces with a pair of large shears as quickly as possible after harvesting and the samples for analysis weighed immediately.

Methods of drying of plant tissue which do not cause a loss of carotene require further investigation before definite recommendations can be made. The work of the present investigation was done largely with machine dried and field dried alfalfa. Machine dried material which had been subjected momentarily to an initial temperature as high as 650° to 750° C., and then to lower temperatures until dry, was found to contain practically the same amount of carotene as fresh alfalfa. On the other hand, the slow drying of silage in a vacuum desiccator over sulphuric acid at room temperature resulted in a loss of 25 per cent. of the carotene present in the fresh material. These findings suggest that a high initial temperature destroys enzymes which might be active in the destruction of carotene at lower temperatures.

### EXTRACTION OF PIGMENTS

The sample of material weighed for analysis should contain about 0.2 mg. of carotene so that final dilution to 100 cc. will give a solution contain-

ing about 0.002 mg. per cc. for use in the colorimeter. If information concerning the approximate percentage of carotene is not available, it is advisable to make preliminary analyses. With samples very low in carotene, better results have been obtained by using a smaller sample than the amount which would contain 0.2 mg., and a correspondingly smaller final volume for the colorimetric comparison. In this investigation the size of samples varied from 1 to 25 gm.

Since carotene was the only pigment to be determined, it was not necessary to isolate chlorophyll and xanthophyll. Dried alfalfa was extracted directly with petroleum ether, because it had been found, in the case of this material, to extract carotene completely while removing only a small amount of the chlorophyll and xanthophyll. In most instances the extraction was carried out by allowing the sample to stand in petroleum ether for 72 hours, with occasional stirring, although for some types of tissue a shorter period of time was sufficient. The volume of petroleum ether varied from 125 to 250 cc., the larger amount being necessary for large samples low in carotene. It was found convenient to carry out the extraction in 250-cc. centrifuge bottles and to separate the plant tissue from the solvent at the end of the extraction period by centrifuging. The finely divided samples did not lend themselves well to filtration, and less solvent was retained by the plant tissue when the separation was made with the centrifuge. The liquid layer was poured or siphoned into a 1-liter separatory funnel, a filter being used as a precaution against the introduction of any of the plant tissue. The extracted tissue was washed with several 50-cc. portions of petroleum ether, five portions with stirring and centrifuging after each addition usually being sufficient. In dealing with new material or different lots of the same material, it was found advisable to test for completeness of extraction by repeating the analytical procedure on the sample which had already been extracted. The amount of carotene obtained by this second extraction determined the length of the extraction period and the number of extractions to be used.

Samples of fresh plant tissue were triturated with sand under acetone and then allowed to stand under the solvent for 48 hours. After decantation through a filter, the residue was washed with several portions of fresh acetone. If the extraction is not complete, as indicated by the removal of all green color from the material, it is necessary to grind the sample further with sand and repeat the extraction. To carry out the remainder of the analysis, the pigments were transferred from acetone to petroleum ether by adding about 400 cc. of the latter solvent to the acetone solution in a separatory funnel. The acetone was then washed out with several 300- to 400-cc. portions of water. Care must be taken in this operation to avoid excess shaking on account of the tendency to form an emulsion.

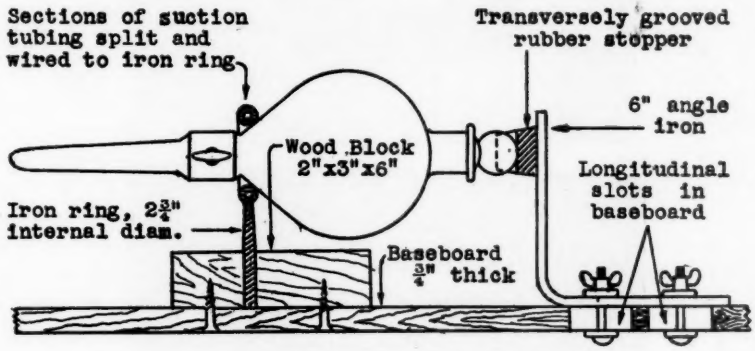
The present study has been concerned almost entirely with alfalfa and spinach plant tissue, and the methods of extracting the plant pigments just described may not apply equally well to other plant tissues. As noted before, SMITH and SMITH (**14**) have employed pyridine for the extraction procedure. Preliminary studies in this laboratory show that pyridine removes from dried carrots and yellow corn an additional amount of carotene, even after they have been thoroughly extracted with petroleum ether. The use of pyridine with alfalfa, however, which had already been extracted with petroleum ether, did not reveal the presence of additional carotene; hence in the application of this method to plant tissue other than alfalfa, special attention should be given to the extraction procedure.

### SEPARATION OF PIGMENTS

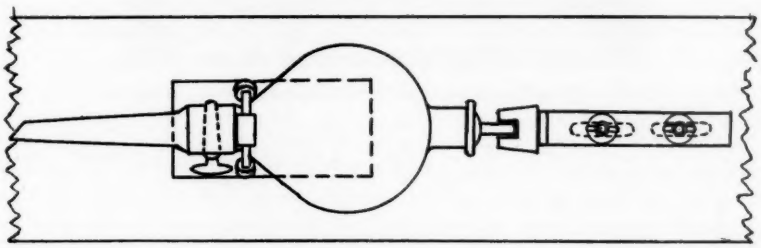
In the case of both dry and fresh samples it is necessary to remove chlorophyll and xanthophyll from the petroleum ether solution. To remove xanthophyll, the solution, having a volume of approximately 400 cc., was shaken in a 1-liter separatory funnel with 50-cc. portions of 89 per cent. methyl alcohol for 2-minute periods. This process was continued until the methyl alcohol layer was only very slightly colored. Usually six extractions were sufficient and at the end of this time only a small amount of xanthophyll still remained in the petroleum ether. However, these last traces of xanthophyll were removed during the process for the removal of chlorophyll. The procedures which involved shaking in a separatory funnel, were facilitated by the use of a shaking machine which had been modified so that it would hold four 1-liter funnels in a horizontal position. Figures 1 and 2 provide a description of the machine. The stopcocks of the funnels were lubricated with stopcock grease and, although there was a slow dissolving of it by the petroleum ether, renewal between every two or three analyses prevented any leakage. The glass stoppers were held so firmly by the pressure of the clamps that there was no leakage. To avoid the possibility that they might become stuck, however, they were lubricated with a soft soap, made by warming together 100 parts of Ivory soap, 100 of water, and 15 of glycerol. This was preferable to stopcock grease which quickly dissolved from around the stopper. It was necessary of course to use lubricants which did not impart a color to the solvents.

Although WILLSTÄTTER and STOLL (**17**) prescribed the use of one portion of 85 per cent. methyl alcohol, one of 90 per cent., and two or more of 92 per cent., it has been the experience in this laboratory that any solution of methyl alcohol greater than 90 per cent. by volume will emulsify if shaken vigorously with petroleum ether; whereas emulsion formation was avoided entirely if the concentration of alcohol was 88 to 89 per cent. by volume.

Chlorophyll was removed by shaking thoroughly the petroleum ether solution with 25 per cent. potassium hydroxide in absolute methyl alcohol. Successive 25-cc. portions of the alcoholic potassium hydroxide were used until no further color was removed. The complete removal of chlorophyll is important at this point since chlorophyll in very low concentrations will impart a slightly yellow color not unlike that due to carotene. In testing for the complete removal of chlorophyll, it was found convenient to have


Sections of suction tubing split and wired to iron ring
Transversely grooved rubber stopper
6" angle iron
Wood Block 2"x3"x6"
Longitudinal slots in baseboard
Iron ring, 2¾" internal diam.
Baseboard ¾" thick

SIDE ELEVATION AND PARTIAL SECTION



PLAN

FIG. 1. Unit of holder for separatory funnels to attach to mechanical shaker.

for comparison a series of very dilute color standards in tubes prepared by diluting the standard potassium dichromate solution. The following concentrations, in terms of carotene equivalents, were employed: 0.01, 0.004, 0.002, 0.001, 0.0004, 0.0002, and 0.0001 mg. per cc. The removal of chlorophyll was considered to be complete when the alcoholic potassium hydroxide extract had a color value equivalent to less than 0.0001 mg. of carotene per cc.

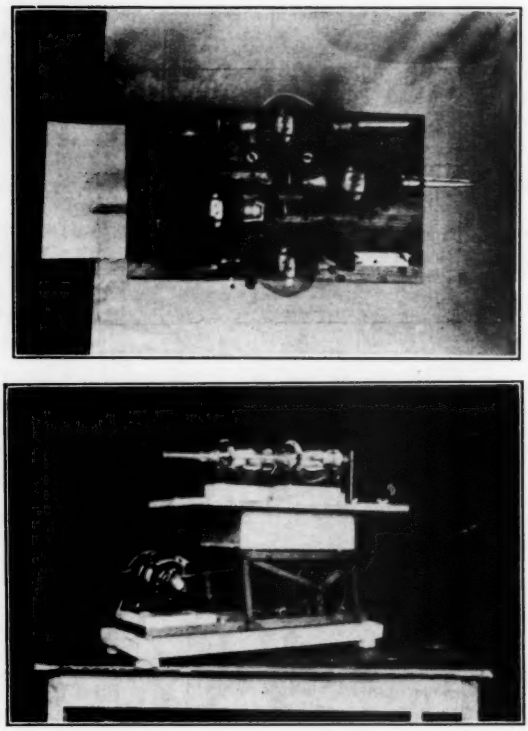


FIG. 2. Assembled apparatus for shaking separatory funnels.

After removal of chlorophyll and xanthophyll, the petroleum ether solution was shaken with 50 cc. of 89 per cent. methyl alcohol, followed by 100 cc. of distilled water in order to remove traces of base. The resulting petroleum ether solution, which contained only carotene, was transferred to a 400-cc. beaker and evaporated to a suitable volume, this process being carried out *in vacuo* at 40°–45°. For this purpose a vacuum desiccator, continuously evacuated with a water pump, was used in a constant temperature oven. When a suitable volume had been obtained, the petroleum ether solution was transferred quantitatively to a volumetric flask, made up to volume with petroleum ether,[2] and compared in a Buerker colorimeter with the standard solution of potassium dichromate, described in a following section.[3]

[2] The use of a high boiling petroleum ether, "Skelleysolve," has been suggested by Prof. R. A. DUTCHER, Pennsylvania State College, in a private communication.

[3] Dr. E. S. MILLER (see addendum) determined the carotene content of a petroleum ether solution prepared by this procedure by spectral analysis and found it to contain 97 per cent. of the carotene content determined colorimetrically in this laboratory.

## Reagents

The acetone and potassium hydroxide were the usual c.p. grade. The petroleum ether was a commercial grade, b.p. 30° to 60°, obtained in 50-gallon drums and redistilled before using. Methyl alcohol was purchased as refined methanol and was sufficiently pure, as indicated by the fact that no color developed in its solution of potassium hydroxide in two weeks. The presence of a cloudiness, presumably potassium carbonate, in the alcoholic solution makes it difficult to determine whether a colorless extract has been obtained in the analytical procedure. The cloudiness was easily removed by centrifuging.

It was found to be economical to recover by distillation the methyl alcohol solution used to remove xanthophyll and to adjust the distillate to the original 89 per cent. concentration.

## Colorimetric procedure

Both spectrophotometric and colorimetric methods have been used by various investigators in estimating the concentration of carotene in the petroleum ether solution obtained by the analytical procedure. The former method has been described by Schertz (**12**) and by Ferrari and Bailey (**4**). However, the error involved in the isolation of carotene is probably as great as that inherent in the colorimetric method, and therefore there seems to be no advantage in the use of the spectrophotometric procedure for which greater accuracy has been claimed (**12**). Recently methods have been proposed, such as that by Oltman (**10**), which involve the measurement of the intensity of transmitted light by means of a photoelectric cell and aim to eliminate the subjective factor inherent in colorimetric readings. To the best of our knowledge, however, methods of this type have not been brought to a wholly satisfactory stage of development.

The reported instability of carotene and the necessity of dissolving it in volatile liquids render it unsuitable as a colorimetric standard. As secondary standards, Willstätter and Stoll (**17**), Palmer (**11**), and Coward (**3**) have made use of potassium dichromate; whereas Sprague and Shive (**15**) availed themselves of a mixture of the dyes Orange G and Naphthol Yellow. Kuhn and Brockmann (**6**) have employed azobenzene. During the early part of this investigation the dye standard described by Sprague and Shive was used, but later it became evident that it possessed no advantage over potassium dichromate, and that the latter was preferable because of its greater uniformity of color and stability in solution.

Both the Orange G-Naphthol Yellow and the potassium dichromate solutions were evaluated against standard carotene solutions. Crystals of the pigment were isolated from dried alfalfa and dried carrots by a process

which is the same in principle as the analytical procedure for dried plant tissue described in detail in previous sections. Three lots of carotene were prepared and the following quantities weighed to the fifth place by the method of swings: carrot carotene lot 1, 39.8 mg.; carrot carotene lot 2, 22.3 mg.; and alfalfa carotene, 18.1 mg. Each sample was brought into solution in slightly less than 200 cc. of petroleum ether with warming to not above 50°, after which the volume was made up to 200 cc. Dilutions of the carotene solutions were prepared which were suitable for comparison with the secondary standards in the region of 10 mm. on the scale of the Buerker colorimeter. The secondary dye standard described by SPRAGUE and SHIVE (**15**) was prepared by diluting 3.4 cc. of 0.5 per cent. Naphthol Yellow and 0.5 cc. of 0.5 per cent. Orange G to 1 liter. For the standard potassium dichromate solution, a concentration of 0.036 per cent. was used because this solution matched the dye standard very closely. Table I

TABLE I

CAROTENE VALUES OF THE SECONDARY STANDARDS

| SOURCE OF CAROTENE | MELTING POINT, CORR. | DYE STANDARD. CAROTENE EQUIVALENT PER CC. | POTASSIUM DICHROMATE STANDARD. CAROTENE EQUIVALENT PER CC. |
|---|---|---|---|
| | °C. | *mg.* | *mg.* |
| Carrot 1 | 175°–177° | 0.00215 | ..... |
| Carrot 2 | 171.5°–175.5° | 0.00227 | 0.00205 |
| Alfalfa | 176.5°–179° | 0.00228 | 0.00210 |
| | Average | 0.00223 | 0.00208 |

shows the carotene values of the two secondary standards. The carotene equivalent of the potassium dichromate solution was adopted as 0.00208 mg. per cc. (See addendum.)

Recent investigations have revealed the existence of two forms of carotene, $\alpha$ and $\beta$ (**5, 8**) and possibly a third, $\gamma$ carotene (**7**). These findings raise the question as to whether the several forms are colorimetrically equivalent. KUHN and BROCKMANN (**6**) have reported that in concentrations of the order of 0.00235 mg. per cc., the $\alpha$ and $\beta$ forms appear to be equal; but that in higher concentrations there is a difference expressed by the proportion $\alpha$ carotene : $\beta$ carotene :: 1.0 : 1.2. The concentration at which they are equivalent is of the same order of magnitude as that employed throughout the present investigation, the 0.036 per cent. potassium dichromate standard solution being equivalent to 0.00208 mg. of carotene per cc. Earlier workers used standards which had a carotene value

several times as high as that just described. Thus WILLSTÄTTER and STOLL (17) and COWARD (3) employed a 0.2 per cent. potassium dichromate solution.

In the use of a colorimetric standard, it is necessary to determine any deviation from Beer's law for a given concentration of standard and type of colorimeter. PALMER (11) has plotted the data obtained by WILLSTÄTTER and STOLL when a Wolff colorimeter was used, and notes that a Dubosq or Kober colorimeter should serve as well. COWARD (3) has prepared a curve for the use of carotene solutions with the Hellige colorimeter. Of several types of colorimeters available, the Buerker was found to be the most satisfactory, probably because it compensates for the use of different solvents in the standard and in the unknown.

To determine the relationship between observed and calculated values over a range of 5 to 20 mm. when the Buerker colorimeter was used, a series of solutions of known concentrations were prepared from alfalfa carotene and from lot 2 of carrot carotene. The solutions were related as 1.5x, x, 0.77x, and 0.57x in the alfalfa carotene series and in the carrot carotene series the relationship was 2x, x, 0.65x, and 0.5x. In the case of each series, concentration x was selected so as to match the standards as nearly as possible at equal depths, that is at 10 units on the scale. The various concentrations of carotene were read in the colorimeter against the secondary standards set at the fixed depth of 10 mm. In table II the observed and calculated colorimeter readings are shown. The latter were calculated upon the assumption that there was no deviation from Beer's law.

Although the differences between the observed and calculated values in table II in most instances fall within the error of colorimetric observation, yet they increase and are positive in character, as the colorimeter reading becomes greater, indicating a deviation from Beer's law. These results and experience with the method in this laboratory lead to the recommendation that readings with the Buerker colorimeter should be made between 7 mm. and 14 mm., with the standard set at 10 mm.

The volatility of the low-boiling petroleum ether used necessitated special precautions in the preparation of solutions of known concentration. To avoid errors from the evaporation incident to pouring, dilution of the most concentrated solution of each series was carried out by measuring the required amounts from a 100-cc. burette into 100-cc. volumetric flasks. The burette was filled from the bottom by suction rather than by pouring, and tilting or agitation of the bottle of concentrated solution was avoided as much as possible. After the preparation of each dilution, the solution remaining in the burette was read colorimetrically and was found not to have undergone any change in concentration. The transfer of the various

## TABLE II

RELATION OF CONCENTRATION TO DEPTH OF COLOR

| RELATIVE CONCENTRATION OF CAROTENE | DYE STANDARD TRIAL 1 | | | DYE STANDARD TRIAL 2 | | | DICHROMATE STANDARD | | |
|---|---|---|---|---|---|---|---|---|---|
| | OBSERVED COLORIMETRIC READING | CALCULATED COLORIMETRIC READING | DIFFERENCE | OBSERVED COLORIMETRIC READING | CALCULATED COLORIMETRIC READING | DIFFERENCE | OBSERVED COLORIMETRIC READING | CALCULATED COLORIMETRIC READING | DIFFERENCE |
| Carrot carotene Lot 2 | *mm.* | *mm.* | *mm.* | *mm.* | *mm.* | *mm.* | *mm.* | *mm.* | *mm.* |
| 2 x | 4.9 | 5.0 | +0.1 | 4.8 | 4.8 | 0.0 | 5.2 | 5.1 | −0.1 |
| x | 9.9 | ..... | ..... | 9.6 | ..... | ..... | 10.3 | ..... | ..... |
| 0.65 x | 15.0 | 15.2 | +0.2 | 14.5 | 14.7 | +0.2 | 15.7 | 15.8 | +0.1 |
| 0.50 x | 19.0 | 19.8 | +0.8 | 18.6 | 19.1 | +0.5 | 20.0 | 20.6 | +0.6 |
| Alfalfa carotene | | | | | | | | | |
| 1.5 x | 6.4 | 6.4 | 0.0 | 6.8 | 6.8 | 0.0 | 7.1 | 7.0 | −0.1 |
| x | 9.5 | ..... | ..... | 10.2 | ..... | ..... | 10.5 | ..... | ..... |
| 0.77 x | 12.4 | 12.4 | 0.0 | 13.3 | 13.2 | −0.1 | 13.6 | 13.7 | +0.1 |
| 0.57 x | 16.3 | 16.7 | +0.4 | 17.6 | 17.9 | +0.3 | 18.1 | 18.5 | +0.4 |

solutions to the colorimeter cup was made by pipetting rather than by pouring.

As has already been stated, the dye standard of SPRAGUE and SHIVE (**15**) was employed in the early stages of this investigation. That the results are essentially the same with both standards is evident from table II, and therefore the dichromate standard was adopted on account of its greater stability and reproducibility. When the dye standard was developed several years ago by SPRAGUE and SHIVE, it was evaluated against a carotene solution, prepared from plant tissue, whose carotene content was determined with a spectrophotometer through the courtesy of Dr. F. M. SCHERTZ of the United States Department of Agriculture. The carotene value of the standard was placed at 0.00189 mg. per cc. As will be noted in table I, the average carotene value obtained by comparison with solutions of three different samples of crystalline carotene is 0.00223 mg. per cc. In view of the more recent findings with regard to carotene and because the solutions used in the present investigation were prepared from crystalline carotene, the later value has been adopted as the carotene value of the dye standard in preference to the earlier one.

### Comparison of petroleum ether with aqueous acetone extraction

In the early stages of this study 85 per cent. aqueous acetone, recommended by WILLSTÄTTER and STOLL (**17**), was used for the extraction of dry plant tissue, prior to the transfer of the pigments to petroleum ether. ARNAUD (**1**) had previously used direct extraction of the pigments with the latter solvent, and this step in the procedure was developed further in this investigation. A comparison of the results obtained by the two procedures is presented in table III, in which it is evident that good agreement prevails between the two methods. Hence direct extraction of dry plant tissue with petroleum ether was adopted because the procedure could be shortened without sacrifice of accuracy.

TABLE III

COMPARISON OF PETROLEUM ETHER WITH AQUEOUS ACETONE EXTRACTION

| SAMPLE | CAROTENE CONTENT, PETROLEUM ETHER EXTRACTION | CAROTENE CONTENT, AQUEOUS ACETONE EXTRACTION |
|---|---|---|
| | % | % |
| Alfalfa 45 | 0.0039 | 0.0040 |
| | 0.0039 | 0.0044 |
| | 0.0040 | 0.0040 |
| | 0.0040 | |
| Spinach 44 | 0.0167 | 0.0175 |
| | 0.0167 | 0.0175 |

## TABLE IV

LOSS OF CAROTENE DUE TO MANIPULATION

| SAMPLE | CAROTENE IN SAMPLE OF TISSUE | CAROTENE ADDED | TOTAL CAROTENE, CALCULATED | CAROTENE FOUND | CAROTENE LOST | LOSS AS PERCENTAGE CAROTENE ADDED |
|---|---|---|---|---|---|---|
| | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | % |
| Alfalfa 45 | 0.188* | 0.183 | 0.371 | 0.362 | 0.009 | 4.9 |
| | | 0.183 | 0.371 | 0.362 | 0.009 | 4.9 |
| | 0.163* | 0.186 | 0.349 | 0.340 | 0.009 | 4.8 |
| | 0.143 | 0.178 | 0.321 | 0.314 | 0.007 | 4.9 |
| Spinach 44 | 0.158* | 0.182 | 0.338 | 0.333 | 0.005 | 2.8 |
| | | 0.182 | 0.338 | 0.333 | 0.005 | 2.8 |
| | 0.161* | 0.182 | 0.343 | 0.335 | 0.008 | 4.5 |
| | | 0.182 | 0.343 | 0.338 | 0.005 | 2.7 |
| | 0.122 | 0.178 | 0.300 | 0.293 | 0.007 | 5.7 |

* Average of duplicate determinations.

### Loss of carotene due to manipulation

To determine whether there was a loss of carotene during the analytical procedure, a solution of crystalline carotene was prepared so that 100 cc. contained an amount of carotene which was approximately the same as that in a sample of plant tissue used for colorimetric analysis. One hundred cc. of this solution was added to a weighed portion of dry plant tissue and the total carotene content determined. At the same time carotene was determined in another sample of the dried plant tissue. The difference between the amount of carotene determined in the prepared sample and that calculated to be present is the amount of carotene lost. In table IV these data and the percentage of pigment lost are shown. Losses of the order of 5 per cent. demonstrate that the destruction of carotene during the analytical procedure is slight and is probably within the range of experimental error.

### Application of analytical procedure

In table V there is shown a series of results obtained upon samples of

TABLE V

ANALYTICAL RESULTS

| Alfalfa 45 | | Alfalfa 61 | | Alfalfa 62 | |
|---|---|---|---|---|---|
| Date | Carotene | Date | Carotene | Date | Carotene |
| | % | | % | | % |
| 1/16/32 | 0.0038 | 4/27/32 | 0.0062 | 4/11/32 | 0.0022 |
| | 0.0041 | | 0.0061 | | 0.0023 |
| | 0.0039 | 5/28/32 | 0.0060 | 4/24/32 | 0.0024 |
| 1/26/32 | 0.0039 | | | | 0.0024 |
| | 0.0040 | | | | |
| | 0.0039 | | | | |
| | | 6/ 2/32 | 0.0063 | | |
| 2/ 5/32 | 0.0041 | 7/19/32 | 0.0059 | 5/27/32 | 0.0021 |
| | 0.0041 | | 0.0057 | | 0.0022 |
| | | 8/ 8/32 | 0.0058 | 7/19/32 | 0.0021 |
| | | | 0.0055 | | 0.0022 |
| Mean | 0.0040 | | 0.0059 | | 0.0022 |
| Coefficient of variation | 0.96 | | 1.5 | | 1.92 |

alfalfa over a period of several months which demonstrate that consistently reproducible results can be obtained with the method described. The material was stored at $0° \pm 5°$.

## Summary

1. The method originally described by WILLSTÄTTER and STOLL, and since modified by others, has been examined critically and revised so as to permit a more complete separation of carotene from other plant pigments.

2. A potassium dichromate solution, of lower color value than that employed by previous investigators, has been evaluated against carotene preparations of known purity and can be used when either the α or β or both forms of carotene are present. (See addendum.)

3. The revised method permits a recovery of 95 to 97 per cent. of added carotene.


*Addendum.*—After this paper was accepted for publication, spectral methods for the determination of α and β carotene were brought to a successful completion by DR. E. S. MILLER, National Research Council Fellow, Department of Chemistry, University of Chicago. (Plant Physiol. 9: 681. 1934.)

Also the development of more refined procedures made possible a supply of α and β carotene. A small quantity of each of these isomers was purchased from the S.M.A. Corporation, Cleveland, Ohio, and portions of the same lot of each were sent to DR. E. S. MILLER and to his laboratory. MILLER determined the purity of the two isomers and found it to be 97 per cent. in each case. The colorimetric value of the potassium dichromate standard was evaluated against each of the isomers in this laboratory. Three separate weighings and solutions were made of each of the isomers. The values observed for the dichromate standard in the case of each of the isomers and their averages are shown in table VI. Although the values when compared with α carotene are generally

TABLE VI

α AND β CAROTENE EQUIVALENTS OF THE POTASSIUM DICHROMATE STANDARD

| CAROTENE SAMPLE | PURITY | CAROTENE EQUIVALENT PER CC. OF POTASSIUM DICHROMATE STANDARD | |
|---|---|---|---|
| | | OBSERVED VALUES | CORRECTED TO 97% PURITY |
| | % | *mg.* | *mg.* |
| Alpha | 97 | 0.00215<br>0.00211 } Av. 0.00215<br>0.00218 | 0.00209 |
| Beta | 97 | 0.00209<br>0.00207 } Av. 0.00209<br>0.00211 | 0.00203 |
| | | | Average 0.0026 |

slightly higher than those for the β form, the difference between the averages of the two sets of readings is within the error commonly accepted for colorimetric work. Therefore it was considered advisable to average the corrected values and to adopt 0.00206 mg. per cc. as the carotene equivalent for the 0.036 per cent. potassium dichromate solution. This value is only slightly different from that originally adopted, 0.00208 mg. per cc. The use of a potassium dichromate standard of the above concentration will permit the

determination of the carotene content of plant tissue when only one or both of the isomers are present. We wish to express to DR. MILLER our appreciation of his critical examination of our paper and of his cooperation in the making of these determinations.

NEW JERSEY AGRICULTURAL EXPERIMENT STATION
NEW BRUNSWICK, NEW JERSEY

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# EFFECT OF SOLAR RADIATION ON TRANSPIRATION OF *HELIANTHUS ANNUUS*

EMMETT V. MARTIN

(WITH FIVE FIGURES)

## Introduction

Considerable attention has been given to radiant energy as a factor in the rate of transpiration. Although the early investigations were for the most part qualitative in character, all demonstrated the strong accelerating action of radiation. BRIGGS and SHANTZ (**4, 5**) have made extensive observations of the daily march of transpiration and environmental factors, and have found a high degree of correlation between water loss and radiation. Recently, ARTHUR and STEWART (**2**) have measured the rate of transpiration of *Nicotiana tabacum* under controlled conditions in the laboratory, and find that to double the radiation increases the rate of water loss approximately 1.7 times. However, an attempt to separate the effect of radiation from that of other environmental factors by means of measurements taken under natural conditions has never been made. From the point of view of the ecologist analyzing the factor complex, such determinations are of considerable value. In an experiment of this sort, it is necessary to change the amount of radiation received by the plants without materially altering the other environmental factors. These conditions were fulfilled in the present work by the use of specially constructed shade tents that transmit different amounts of radiation but allow such freedom of air circulation that the other factors are not appreciably affected.

## Methods

In these experiments two types of shade tents were employed. The first consisted of a plane framework of wood 3 feet square covered with cloth, the weave and number of layers determining the intensity of radiation transmitted. These screens were supported at a distance of 1 to 2 feet from the plants and were moved every few minutes during a series so as to maintain the plants in shadow. The second type was somewhat more elaborate in design, as shown in figure 1. The main arch is a 20-foot piece of 3-inch redwood batten bent into the form of a semicircle, while the lower arch is 10 feet long and of similar curvature. The centers of these two pieces are connected by a 4-foot piece of $\frac{1}{2}$-inch iron rod and their ends by 6-foot pieces of batten. This structure is supported with the main arch in an east-west line by five stakes driven in the ground, one at each end of the 6-foot crosspieces and one at the center of the lower arch.

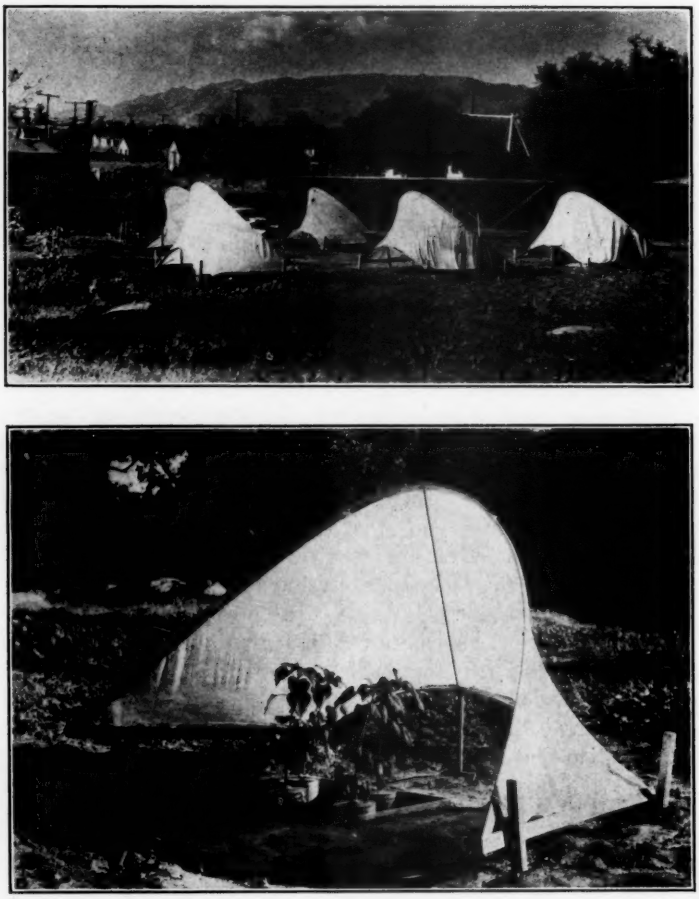

FIG. 1. Above, distant view of entire group of tents; below, close-up view of tent *D*.

Five of these tents were used, each covered with a different type and thickness of cloth as follows: *B* with one layer of cheesecloth with a thread count of $20 \times 16$; *C* with one $28 \times 24$; *D* with one each of $20 \times 16$ and $36 \times 32$; *E* with one layer of sheeting $56 \times 60$; and *F* with two layers of medium-weight white canvas. The iron rod across the center of each tent was removable, thus permitting the tents to collapse when not in use, a feature that was found to be necessary for even moderately windy days. A wooden box, 4 feet long, 2 feet wide, and 1 foot deep, to hold the phy-

tometers, was sunk in the ground in the center of the space covered by the tent, with its long side in an east-west line. During the fall and winter months, these tents cast the desired shadow all day; but for use in summer, they would need to be constructed differently.

For some of the series, plants of *Helianthus annuus* were grown from seed in water-tight, cylindric, galvanized iron cans 8 inches in diameter and 10 inches in height, while for others similar cans $8\frac{1}{2} \times 11$ inches were employed. Each of these was fitted with a removable lid that had in its center a circular opening 2 inches in diameter. The soil used was a good loam of as nearly uniform texture and moisture as was feasible. The holard was set at approximately 65 per cent. of saturation (or 28 per cent. of the dry weight of the soil), and the total amount of water in each can was kept within 15 per cent. of this value throughout the growing period; and during all series it was maintained constant within 3 per cent. When necessary, water was added through a glass tube extending into a layer of gravel about 1 inch thick in the bottom of the cans. When the plants were a few weeks old, the containers were sealed by adding a layer of sand about $\frac{3}{4}$ inch thick on top of the soil, filling the opening in the lid with non-absorbent cotton, and corking the glass tube. The efficiency of this type of seal was tested by means of controls without plants. Weighings made on a torsion balance to the nearest gram during the time the experiments were in progress showed that such containers seldom lost a detectable amount. This seal has an advantage over the paraffin type in that forced aeration is unnecessary (CLEMENTS and GOLDSMITH **6**).

In all series, six sets of from four to six plants each were used, one set under each tent and a control group in the open, the plants in each set being arranged so as not to shade one another. For the whole-day series the plants were placed under the tents before sunrise and weighed every hour throughout the day. For the short series the plants were put in position about half an hour before the first weighing, which was usually about ten or eleven o'clock in the morning, allowed to remain undisturbed for two to four hours (the time depending upon weather conditions), and again weighed. On account of the fact that clouds cause such rapid fluctuations in radiant energy, series could be run only during periods of cloudless skies. Measurements of air temperature, relative humidity, and average wind velocity were taken every hour during all series. Leaf areas in square decimeters were determined by multiplying the product of the length and width of the leaves in centimeters by the factor 0.0134 (**6**).

Measurements of solar radiation intensity under each tent and in the open were taken two or three times each hour by means of the Smithsonian pyranometer (**1**), which records the intensity of radiation (direct plus diffuse) falling on a horizontal surface. The average intensity during a series

was determined by plotting the observed values on cross-section paper, connecting consecutive points by straight lines as in figure 3, reading the values from this graph for every ten or fifteen minutes, and averaging them.

## Results

The transpiration data and radiation measurements for the whole-day series of November 2, 1934, are given in tables I and II respectively, and

TABLE I

TRANSPIRATION DATA OF NOVEMBER 2, 1934

| TIME | | TENT | | | | | |
|---|---|---|---|---|---|---|---|
| | | A (CONTROL) | B | C | D | E | F |
| A. M. | W*= | 30 | 27 | 47 | 20 | 25 | 23 |
| 7–8 | t = | 0.98 | 0.97 | 0.95 | 0.93 | 0.90 | 0.87 |
| | T = | 0.27 | 0.27 | 0.41 | 0.19 | 0.28 | 0.27 |
| | W = | 118 | 97 | 105 | 67 | 64 | 54 |
| 8–9 | t = | 0.90 | 0.92 | 0.90 | 0.90 | 0.90 | 0.92 |
| | T = | 1.14 | 1.02 | 0.96 | 0.86 | 0.71 | 0.59 |
| | W = | 203 | 136 | 154 | 109 | 83 | 65 |
| 9–10 | t = | 1.00 | 0.98 | 0.98 | 0.97 | 0.98 | 0.97 |
| | T = | 1.77 | 1.35 | 1.30 | 0.99 | 0.85 | 0.68 |
| | W = | 252 | 198 | 205 | 161 | 131 | 103 |
| 10–11 | t = | 1.03 | 1.05 | 1.08 | 1.10 | 1.08 | 1.10 |
| | T = | 2.12 | 1.83 | 1.57 | 1.30 | 1.21 | 0.95 |
| | W = | 270 | 220 | 229 | 183 | 145 | 115 |
| 11–12 | t = | 1.02 | 1.02 | 1.00 | 1.00 | 1.00 | 1.00 |
| | T = | 2.30 | 2.09 | 1.89 | 1.62 | 1.45 | 1.16 |
| P. M. | W = | 323 | 253 | 269 | 225 | 177 | 148 |
| 12–1 | t = | 1.18 | 1.18 | 1.18 | 1.18 | 1.18 | 1.18 |
| | T = | 2.38 | 2.08 | 1.88 | 1.69 | 1.50 | 1.27 |
| | W = | 224 | 189 | 199 | 161 | 128 | 105 |
| 1–2 | t = | 0.88 | 0.87 | 0.85 | 0.83 | 0.82 | 0.82 |
| | T = | 2.22 | 2.11 | 1.94 | 1.72 | 1.56 | 1.29 |
| | W = | 275 | 233 | 257 | 213 | 174 | 142 |
| 2–3 | t = | 1.02 | 1.02 | 1.03 | 1.05 | 1.08 | 1.12 |
| | T = | 2.34 | 2.22 | 2.06 | 1.80 | 1.61 | 1.28 |
| | W = | 222 | 179 | 179 | 153 | 110 | 90 |
| 3–4 | t = | 1.02 | 1.02 | 1.02 | 1.03 | 1.03 | 0.98 |
| | T = | 1.89 | 1.70 | 1.45 | 1.31 | 1.07 | 0.93 |
| | W = | 81 | 60 | 63 | 52 | 42 | 33 |
| 4–5 | t = | 0.95 | 0.93 | 0.95 | 0.93 | 0.90 | 0.93 |
| | T = | 0.74 | 0.63 | 0.55 | 0.50 | 0.47 | 0.36 |

* W, water loss in grams per set of five plants; t, time interval between weighings in hours; T, transpiration rate in grams per square decimeter leaf area per hour. The leaf areas in square decimeters for the six sets are: A = 115, B = 103, C = 121, D = 113, E = 100, and F = 99.

## TABLE II

MEASUREMENTS OF TOTAL RADIATION INTENSITY ON NOVEMBER 2, 1934*

| TIME | TENT | | | | | |
|---|---|---|---|---|---|---|
| | A (CONTROL) | B | C | D | E | F |
| 7: 00– 7: 06 | 0.032 | 0.032 | 0.029 | 0.029 | 0.028 | 0.023 |
| 7: 57– 8: 05 | 0.413 | 0.239 | 0.275 | 0.080 | 0.092 | 0.044 |
| 8: 29– 8: 38 | 0.557 | 0.441 | 0.394 | 0.216 | 0.119 | 0.049 |
| 8: 50– 8: 59 | 0.652 | 0.487 | 0.440 | 0.251 | 0.136 | 0.051 |
| 9: 24– 9: 32 | 0.77 | 0.607 | 0.492 | 0.315 | 0.151 | 0.054 |
| 9: 52– 9: 59 | 0.83 | 0.65 | 0.537 | 0.314 | 0.156 | 0.054 |
| 10: 22–10: 32 | 0.93 | 0.73 | 0.571 | 0.349 | 0.167 | 0.057 |
| 10: 52–11: 00 | 0.99 | 0.77 | 0.66 | 0.371 | 0.177 | 0.061 |
| 11: 29–11: 37 | 1.02 | 0.78 | 0.67 | 0.372 | 0.182 | 0.061 |
| 11: 52–12: 01 | 1.01 | 0.74 | 0.66 | 0.396 | 0.172 | 0.061 |
| 12: 31–12: 38 | 0.99 | 0.73 | 0.60 | 0.406 | 0.168 | 0.061 |
| 1: 05– 1: 14 | 0.88 | 0.70 | 0.548 | 0.381 | 0.158 | 0.061 |
| 1: 41– 1: 48 | 0.81 | 0.583 | 0.504 | 0.354 | 0.146 | 0.061 |
| 1: 59– 2: 05 | 0.77 | 0.573 | 0.480 | 0.342 | 0.139 | 0.058 |
| 2: 28– 2: 37 | 0.68 | 0.494 | 0.405 | 0.273 | 0.138 | 0.051 |
| 2: 58– 3: 07 | 0.568 | 0.407 | 0.355 | 0.244 | 0.122 | 0.044 |
| 3: 37– 3: 43 | 0.360 | 0.248 | 0.204 | 0.163 | 0.091 | 0.039 |
| 4: 03– 4: 08 | 0.216 | 0.168 | 0.126 | 0.117 | 0.065 | 0.032 |
| 4: 35– 4: 41 | 0.037 | 0.037 | 0.031 | 0.055 | 0.032 | 0.021 |
| 5: 01– | 0.008 | ..... | ..... | ..... | ..... | ..... |

* Values of total radiation intensity are in calories per square centimeter per minute.

are represented graphically in figures 2 and 3. In both figures curve A

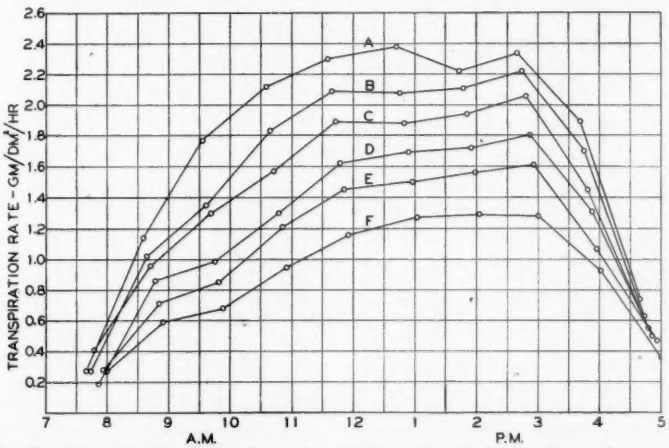


FIG. 2. Transpiration rates for series of November 2, 1934: Curve A corresponds to the open-air control and curves B to F to tents *B* to *F* respectively.

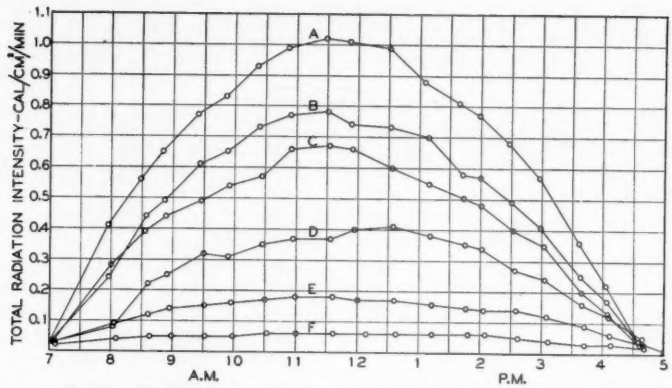


FIG. 3. Total radiation intensities for series of November 2, 1934: Curve A corresponds to the open-air control and curves B to F to tents *B* to *F* respectively.

corresponds to the open-air control and curves B to F to tents *B* to *F* respectively. The transpiration curves for all six conditions are similar in the course of their daily march, but the absolute values decrease with lower radiation. The daily march is controlled by the variations in radiation and meteorological factors throughout the day; but while the radiation differs considerably for the various tents, the other factors are practically identical. Air temperature in the open rarely varied more than 1° from that under tent *F*, as can be seen in table III, while the averages for the two agreed very closely. The construction of the tents being such as to permit fairly free circulation of air, relative humidity and wind velocity

TABLE III

METEOROLOGICAL DATA FOR NOVEMBER 2, 1934

| | TIME OF DAY | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 7 | 8 | 9 | 10 | 11 | 12 | 1 | 2 | 3 | 4 | 5 |
| Air temperature (open)* | 50 | 61 | 67 | 68 | 70 | 72 | 74 | 75 | 76 | 75 | 68 |
| Air temperature (tent *F*) | 50 | 61 | 67 | 68 | 71 | 73 | 75 | 75 | 75 | 74 | 67 |
| Relative humidity | 80 | 58 | 45 | 53 | 40 | 46 | 43 | 40 | 34 | 30 | 46 |
| Wind velocity | ..... | 0.1 | 0.1 | 0.5 | 3.3 | 3.8 | 4.8 | 3.3 | 3.8 | 4.8 | 0.6 |

* Air temperatures are in ° F.; relative humidity in percentage; wind velocity in miles per hour. Each value of wind velocity is the average for the hour preceding the time of day for which it is given.

## TABLE IV

RADIATION AND TRANSPIRATION DATA FOR SHORT SERIES

| DATE | TIME LIMITS | | TENT | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | A (CONTROL) | B | C | D | E | F |
| 4/21/33 | 10:00–2:30 | R* = | 1.36 | 1.04 | 0.81 | 0.576 | 0.343 | 0.144 |
| | | T = | 1.76 | 1.43 | 1.18 | 1.01 | 0.66 | 0.55 |
| | | $r_T$ = | ± 0.035 | ± 0.060 | ± 0.034 | ± 0.049 | ± 0.040 | ± 0.020 |
| 5/13/33 | 12:15–3:00 | R = | 1.32 | 1.00 | 0.82 | 0.550 | 0.355 | 0.143 |
| | | T = | 1.66 | 1.29 | 1.21 | 1.07 | 0.93 | 0.66 |
| | | $r_T$ = | ± 0.044 | ± 0.038 | ± 0.032 | ± 0.041 | ± 0.020 | ± 0.031 |
| 3/27/34 | 10:45–3:00 | R = | 1.09 | 0.74 | 0.61 | 0.454 | 0.257 | 0.130 |
| | | T = | 2.21 | 1.69 | 1.58 | 1.38 | 1.17 | 0.93 |
| | | $r_T$ = | ± 0.032 | ± 0.029 | ± 0.038 | ± 0.021 | ± 0.022 | ± 0.020 |
| 3/31/34 | 10:30–3:00 | R = | 1.26 | 0.87 | 0.75 | 0.475 | 0.273 | 0.082 |
| | | T = | 2.74 | 2.41 | 2.32 | 1.92 | 1.63 | 1.34 |
| | | $r_T$ = | ± 0.061 | ± 0.062 | ± 0.071 | ± 0.046 | ± 0.059 | ± 0.035 |
| 8/1/34 | 9:15–11:00 | R = | 1.45 | 1.15 | 0.96 | 0.75 | 0.529 | 0.058 |
| | | T = | 2.12 | 1.95 | 1.85 | 1.61 | 1.36 | 0.99 |
| | | $r_T$ = | ± 0.076 | ± 0.037 | ± 0.036 | ± 0.045 | ± 0.021 | ± 0.034 |
| 8/9/34 | 10:30–1:30 | R = | 1.40 | 1.13 | 0.79 | 0.494 | 0.205 | 0.061 |
| | | T = | 4.90 | 4.70 | 4.45 | 3.92 | 3.30 | 3.14 |
| | | $r_T$ = | ± 0.112 | ± 0.132 | ± 0.107 | ± 0.026 | ± 0.133 | ± 0.098 |
| 8/11/34 | 10:00–11:45 | R = | 1.39 | 1.06 | 0.71 | 0.415 | 0.208 | 0.086 |
| | | T = | 3.86 | 3.61 | 3.01 | 2.75 | 2.69 | 2.58 |
| | | $r_T$ = | ± 0.031 | ± 0.175 | ± 0.108 | ± 0.021 | ± 0.058 | ± 0.078 |
| 10/11/34 | 10:15–3:15 | R = | 0.94 | 0.72 | 0.570 | 0.374 | 0.198 | 0.088 |
| | | T = | 2.75 | 2.28 | 2.09 | 1.90 | 1.62 | 1.33 |
| | | $r_T$ = | ± 0.045 | ± 0.028 | ± 0.043 | ± 0.031 | ± 0.039 | ± 0.032 |
| 10/22/34 | 11:00–3:15 | R = | 0.93 | 0.63 | 0.592 | 0.348 | 0.157 | 0.059 |
| | | T = | 2.23 | 1.77 | 1.85 | 1.40 | 1.16 | 1.08 |
| | | $r_T$ = | ± 0.115 | ± 0.045 | ± 0.055 | ± 0.042 | ± 0.036 | ± 0.051 |
| 11/2/34 | 11:00–3:15 | R = | 0.87 | 0.65 | 0.526 | 0.345 | 0.150 | 0.055 |
| | | T = | 2.31 | 2.13 | 1.94 | 1.71 | 1.53 | 1.25 |
| | | $r_T$ = | ± 0.040 | ± 0.009 | ± 0.032 | ± 0.051 | ± 0.033 | ± 0.037 |

*R, average radiation intensity in calories per square centimeter per minute for period between weighings; T, average transpiration rate in grams per square decimeter per hour for same period; $r_T$, probable error in T calculated from values given by the various individuals in each set of plants (number of plants in each set is shown in table V).

likewise showed very little difference, so that only the values of these factors obtained from measurements in the open are given in the table.

The transpiration and radiation data for the short series are recorded in table IV, while meteorological and other data are included in table V. The series of August 1 was run at Windy Point, elevation 12,000 feet, on the slopes of Pike's Peak; those of August 9 and 11 at Colorado Springs, Colorado, elevation 6000 feet; and all others at Santa Barbara, California, practically at sea level. The results from five of these series are shown graphically in figure 4, which portrays transpiration rate as a function of

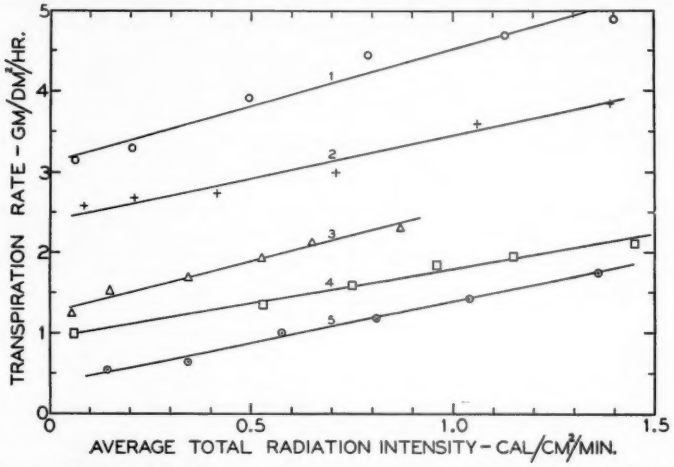


FIG. 4. Results of short series of April 21, 1933 (5), August 1, 1934 (4), August 9, 1934 (1), August 11, 1934 (2), and November 2, 1934 (3).

radiation intensity. Curve 1 represents the data of August 9, curve 2 those of August 11, curve 3 those of November 2, curve 4 those of August 1, and curve 5 those of April 21. In all cases, not only does the rate of transpiration increase with higher intensity of radiation, but the rate of increase is linear. Accordingly the data from all short series are best represented graphically by straight lines, or mathematically by equations of the type $T = b + mR$, where T is the rate of transpiration, R the radiation intensity, b the intercept on the T axis, and m the slope of the straight line. From the point of view of the plant, b should represent the transpiration rate as determined by the combined action of all factors other than radiation, while m should be a measure of the direct effect of the latter.

The method of least squares (various texts, and BIRGE 3) was employed in obtaining from the various series the values of b and m and their probable errors (table V). Both m and b were found to vary considerably for

the different series. Since b is influenced by several factors, it does not show a high degree of correlation with any particular one. In general, however, it increases with higher evaporating power of the air; m, on the other hand, does not vary in the same manner as b. This is readily discernible from table V, but is also clearly shown by the data from the whole-day series of November 2. If the average transpiration rates and radiation intensities for the period 8–9 are used, the value of b is found to be $0.406 \pm 0.012$ and that of m is $1.203 \pm 0.037$. Similarly, the data for the period 12–1 yield $b = 1.200 \pm 0.027$ and $m = 1.194 \pm 0.046$. The former is distinctly different for the two periods, while the latter remains constant; hence m is apparently not affected by changes in environmental factors such as take place during the day. On this basis the observed changes in m for different days must be ascribed to some factor or factors in which changes from day to day take place. Two outstanding examples of these are size and age of plants. There appears to be no correlation between average leaf area and m, but there does seem to be a definite relation with age, which is graphically shown in figure 5. Both m and the rate of change of m with age decrease with increasing age. Of the ten points plotted,

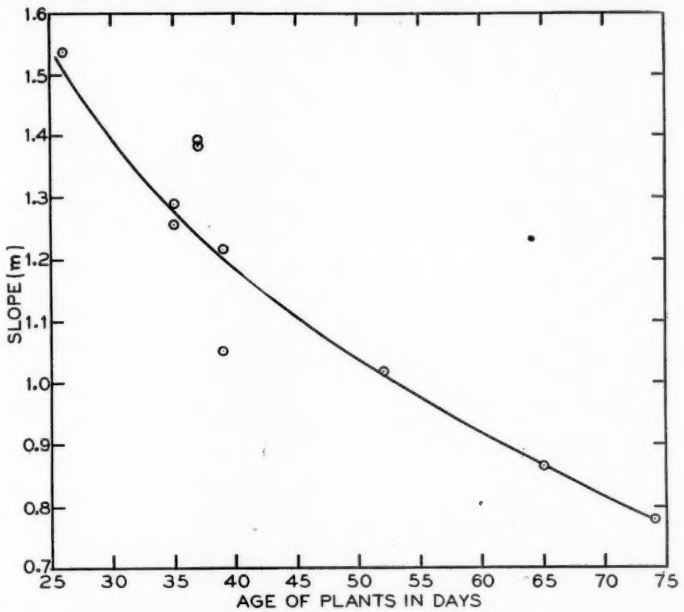


FIG. 5. Slope of transpiration rate *vs.* radiation intensity curves as a function of age of plants in days.

only two are off the curve by more than their probable errors, and these by only twice that amount.

An indication of the importance of radiant energy as a factor in transpiration is furnished by the ratio $mR/(mR + b)$, which gives the fraction of the rate of water loss that is ascribable to the direct effect of radiation. Since this ratio is influenced by a number of factors, it varies over a wide range of values and is usually larger on cold humid days than on warm dry ones. Under the conditions in these experiments, it varied from 38 to 81 per cent., but under more extreme conditions a greater range could be expected.

Leaf temperatures of the plants in the open and under tent *F* were taken at noon for three of the series by means of the well known thermocouple method (**7**). The temperature of leaves hanging nearly at right angles to the sun's rays in the open averaged about 1° C. above that of the air, while that of leaves under tent *F* was about 5° C. below. These temperatures, however, fluctuated so much with air currents and for different leaves on the same plant that no attempt was made to obtain the average for entire plants under the various conditions.

### Accuracy of results

The designers of the pyranometer (**1**) claim that it is accurate to 1 per cent. However, the method used in the present work to obtain the average over a period of a few hours introduces another factor of uncertainty, which can only be estimated; but it seems conservative enough to place the probable error in the average intensities given in table IV at from 2 to 3 per cent.

Errors in the measurements of the rate of transpiration may be grouped into two classes, accidental and constant or systematic. The former includes errors in weighing, in measurements of leaf areas, and those caused by structural differences in the plants themselves. Since these will appear in the results as variations among the individuals in each set of plants, an estimation of their magnitude is furnished by the probable errors in the values of T. These were calculated by the method of least squares from the individual values that were averaged to obtain T and are given in table IV as $r_T$. Expressed in percentage of T, these range from 0.4 to 6.1 with an average of 2.7 per cent.

An estimation of the accuracy with which the data are represented by equations of the type $T = b + mR$ was obtained from the deviations of the observed values of T from those calculated from these equations with the appropriate values of m and b. Expressed in percentage of the calculated values, these deviations range from 0 to 8.3 with an average of 3.0 per cent. Since these are of the same order of magnitude as the probable errors in T, the relation between rate of transpiration and intensity of radiation can

be considered linear within the limits of experimental error. These deviations are responsible for uncertainty in the values of b and m, given in table V with their probable errors which in the case of b range from 2.2 to 6.2 with an average of 3.5 per cent., and in the case of m from 2.8 to 7.0 with an average of 4.8 per cent.

The second class of errors (constant or systematic) includes those caused by such factors as stomatal opening, time required to weigh the entire group of plants, and differences in the quality of radiation under the various tents. The degree of opening of the stomata of each set of plants was determined at hourly intervals during the whole-day series and most of the short ones by means of the alcohol method (**10**), but at no time was any appreciable difference found. The uncertainty introduced by the time taken to weigh the entire group of plants (20 to 25 minutes) has been discussed in an earlier paper (**7**) and shown to be negligible under the conditions of these experiments.

IVANOV and THIELMAN (**9**) have shown that the rate of transpiration of potted plants may be 50–60 per cent. greater under blue-violet than under red-yellow light of the same intensity. Accordingly this factor must be considered as a possible source of error in these investigations, since there is a gradual shift in the wave length of maximum intensity in passing from open air condition *A* to tent *F*. The plants in the open receive full sunlight, while those under tent *F* get only the light reflected from nearby objects and that from the north sky with its greater proportion of short wave lengths. The operation of this factor would tend to increase the transpiration rates in the lower range of intensity, which would appear in the results in the form of larger values of b and smaller values of m than would be obtained with no differences in quality.

## Discussion

LECLERC DU SABLON (**11**) has suggested that the accelerating effect of radiation on transpiration may be due partly to heating of the leaves and partly to a change in the permeability of the protoplasm. Confirmation of the second part of this hypothesis has been furnished by IVANOV and THIELMAN (**9**), who have shown that the transpiration of potted plants may be 50–60 per cent. greater under blue-violet light than under red-yellow light of the same intensity. They suggest that this effect is due to a greater influence of the blue-violet on the permeability of the protoplasm. LEPESCHKIN (**12**) has shown that the permeability of the protoplasm of *Elodea* leaves to methylene blue increases with increase of light up to 10 per cent. of full sunlight, but for intensities above this there is very little, if any, change. Since practically all the intensities used in the present work are above this value, it may be that change of permeability plays a

## TABLE V

ADDITIONAL DATA FOR SHORT SERIES

| DATE | AV. AIR TEMP. | AV. REL. HUMIDITY | AV. WIND VELOCITY | AIR PRESSURE | AGE OF PLANTS | AV. LEAF AREA PER PLANT | TYPE OF TENT | PLANTS PER SET | b* | m* |
|---|---|---|---|---|---|---|---|---|---|---|
| | *°F.* | *%* | *m.p.h.* | *cm. Hg* | *days* | *dm.²* | | | | |
| 4/21/33 | 66 | 51 | 1.8 | 76 | 52 | 11.9 | Sq. | 4 | 0.372 ± 0.023 | 1.018 ± 0.029 |
| 5/13/33 | 64 | 53 | 2.6 | 76 | 74 | 14.1 | Sq. | 4 | 0.595 ± 0.034 | 0.779 ± 0.043 |
| 3/27/34 | 71 | 62 | 1.9 | 76 | 35 | 13.2 | Arc | 6 | 0.808 ± 0.023 | 1.255 ± 0.036 |
| 3/31/34 | 74 | 41 | 1.7 | 76 | 39 | 18.6 | Arc | 6 | 1.306 ± 0.042 | 1.216 ± 0.058 |
| 8/ 1/34 | 62 | 41 | 2.0 | 49 | 65 | 6.0 | Sq. | 4 | 0.937 ± 0.034 | 0.865 ± 0.036 |
| 8/ 9/34 | 86 | 31 | 7.6 | 61 | 37 | 5.7 | Sq. | 4 | 3.123 ± 0.081 | 1.393 ± 0.097 |
| 8/11/34 | 82 | 41 | 1.8 | 61 | 39 | 6.8 | Sq. | 4 | 2.402 ± 0.053 | 1.051 ± 0.067 |
| 10/11/34 | 76 | 53 | 1.6 | 76 | 26 | 6.8 | Arc | 6 | 1.254 ± 0.041 | 1.534 ± 0.073 |
| 10/22/34 | 73 | 48 | 1.6 | 76 | 37 | 25.3 | Arc | 4 | 0.956 ± 0.029 | 1.383 ± 0.054 |
| 11/ 2/34 | 73 | 41 | 3.9 | 76 | 35 | 21.7 | Arc | 5 | 1.254 ± 0.034 | 1.289 ± 0.066 |

* Values of b and m given are accompanied by their probable errors calculated by the method of least squares.

minor rôle in the results, and that the accelerating action of radiation observed is due largely to its heating effect. No estimate of the relative magnitude of the effects of these two factors was obtained in these experiments, since it would require knowledge of air and leaf temperature differences as well as of the amount of radiant energy absorbed by the leaves, both of which are much too variable under natural conditions to be measured accurately. The fact that the relation between radiation and transpiration is linear, however, indicates that any change of permeability that may exist under the conditions in these tests will be either small in comparison to the heating effect or directly proportional to the intensity of radiation.

Similarly, the cause or causes of the variation of the accelerating effect of radiation with age of the plants (fig. 5) cannot be determined from the results of these investigations. Since the slope of the straight lines (m) represents the direct effect of radiation on the transpiration rate, the decrease that occurs in it with increasing age of the plants must be associated with changes in the influence of radiation. If the latter is of a twofold nature, as already mentioned, these changes in m may be brought about in either of two ways. The changes that take place in the leaves as they grow older may be such that the coefficient of absorption for radiation is reduced so that the heating effect is less pronounced, or they may be such that the influence of radiation on the permeability of the protoplasm becomes less effective with increasing age.

## Summary

1. The influence of radiation on the rate of transpiration of *Helianthus annuus* has been measured under natural conditions by the use of shade tents constructed so as to transmit different amounts of radiation and to permit such freedom of air circulation that other environmental factors were not altered appreciably.

2. The ten series made covered a rather wide range of conditions, but in all cases the relation between radiation intensity and transpiration rate was found to be linear within the limits of experimental error.

3. The results of all series could be satisfactorily represented by equations of the type $T = b + mR$, where T is the transpiration rate, R the radiation intensity, b the intercept on the T axis, and m the slope of the straight line.

4. It was found that b varied directly with the evaporating power of the air and m inversely with the age of the plants.

5. The fraction of the transpiration rate due to the direct effect of radiation is given by the ratio $mR/(mR + b)$, and for plants in full sunlight was found to vary from 38 to 81 per cent., depending largely upon the evaporating power of the air.

6. Calculations of probable errors indicate that on the average the results are accurate to within 5 per cent.

CARNEGIE INSTITUTION OF WASHINGTON
SANTA BARBARA, CALIFORNIA

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# METHOD FOR QUICKLY DETERMINING NITROGEN IN PLANTS, AND SOLUBLE NITROGEN AS A MEASURE OF THE NITROGEN AVAILABLE FOR ANABOLIC PROCESSES[1]

E. M. Emmert

## Part I: Determination of total nitrogen

In previous papers (**1**) a method was described for determining total nitrogen by oxidizing with sodium chlorate and sulphuric acid, distilling and determining nitric acid in the distillate by the phenoldisulphonic acid method. This procedure, although more rapid than the standard Kjeldahl method, requires considerable time and equipment, and the technique is rather exacting. Recent work has shown that, by regulating conditions properly, distillation can be dispensed with and the desired result accomplished by adding phenoldisulphonic acid directly to the mixture after oxidation.

### Procedure

Put 100 to 300 mg. of finely ground, well averaged, dry tissue or 500–1500 mg. of green tissue into a 200-cc. Erlenmeyer flask. There should be at least 0.5 mg. of nitrogen in the sample used. Add 1 gm. of sodium chlorate for each 100 mg. of dry sample or each 500 mg. of green sample. Add 25 cc. of 50 per cent. by volume sulphuric acid and attach the flask to a water-cooled reflux condenser. Heat with a high flame until the oxidation is complete and all chlorine color has disappeared from the solution (usually 3 to 5 minutes). Remove the flame, flush out the condenser with 10–15 cc. of 50 per cent. sulphuric acid, cool somewhat (a cold water bath may be used), detach from the condenser and make the solution to 50 cc. with 50 per cent. sulphuric acid. Immediately put exactly 1 or 2 cc. of the solution into a suitable graduated flask, add 3 or 6 cc. of phenoldisulphonic acid, mix well, add about 20 cc. of water, make alkaline with 40 per cent. NaOH solution, adding it until the maximum yellow color is produced and make to volume. After thorough mixing, the clear solution is compared with a standard in a colorimeter. The volume of the solution should be such that the color is fairly close to that of the standard.

### Notes

1. In low nitrogen samples larger aliquots may be used, but the phenoldisulphonic acid should be increased in proportion. It may be advan-

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

tageous to keep the amount of acid lower so that the dilution can be less than 50 cc., although too little acid should not be used since the efficiency of the oxidation may be reduced.

2. If the solution after phenoldisulphonic acid addition is yellow, the decomposition of chloric acid was not complete and the heating was not continued long enough.

3. Most of the chlorine is expelled as chlorine gas, but some chloride is left in the acid solution. When the aliquot was small and the phenoldisulphonic acid was added to the 50 per cent. acid solution immediately after the oxidation instead of to dry salts, no loss of nitrate could be detected. A standard nitrate solution was treated with chlorate as described in the procedure and no loss was detected, even in larger aliquots, when the phenoldisulphonic acid was added to the 50 per cent. acid solution. The presence of a small amount of water to keep the salts in solution overcomes the loss caused by chloride, which would be experienced if phenoldisulphonic acid were applied to dry salts. Evidently nitrosyl chloride is not formed in the presence of water, and as long as the acid is not diluted too much the reaction between nitrate and phenoldisulphonic acid is complete.

4. Presence of much iron, manganese, or perhaps some other heavy metals causes loss of nitrate nitrogen. Error from this source is not appreciable in the analysis of normal plant tissues since the amount of heavy metals in relation to the nitrogen present is very small. In soils and some fertilizers, however, enough heavy metal may be present to cause loss. The writer has devised a procedure for soils (5) in which the metals are precipitated in the presence of excess oxidizing agent, before adding phenoldisulphonic acid.

5. The reaction with chlorate usually proceeds smoothly without the formation of chlorine dioxide. If green fumes of chlorine dioxide should start accumulating it is usually because too low a flame was used and the air in the flask above the solution was not raised to over 100° before the solution got hot. Even then, with the small amount of chlorate used, chlorine dioxide seldom accumulates enough to cause a serious explosion.

6. A round-bottom flask should not be used since the reaction is more likely to be violent. The wide bottom of an Erlenmeyer spreads out the solution and does not allow action to be concentrated at one point. Reflux of water usually washes back any particles of sample which stick to the sides of the flask. If a high flame is used the solution boils up enough to reach most particles on the sides of the flask.

7. On cooling, sodium sulphate may crystallize out of the neutralized yellow solution, especially if the volume is small. For accurate work it is

best not to remove these crystals, since the hydration of the crystallized salts tends to concentrate the solution and cause a high result. It is best to warm the solution a little or keep it warm enough to prevent crystallization. In fact, the most accurate colorimetric readings are obtained immediately after dilution to the final volume, before the solution has a chance to change temperature very much and cause slight volume changes or crystallization of salts.

## RESULTS

Tables I and II show the results obtained on some plant tissues of different kinds. The Kjeldahl results were obtained by the chemistry, agronomy, and feed control departments of the Kentucky Experiment Station in their regular routine work. These results were taken as correct

TABLE I

TOTAL NITROGEN FOUND BY THE KJELDAHL AND CHLORATE METHODS (PERCENTAGE OF DRY TISSUE)

| SAMPLE | KJELDAHL METHOD | CHLORATE METHOD | PERCENTAGE ERROR |
|---|---|---|---|
| | % | % | % |
| Tobacco | | | |
| Leaves (cured) | 2.85 | 2.84 | −0.35 |
| Young plants | 4.35 | 4.35 | 0 |
| Medium aged plants | 3.96 | 3.98 | +0.51 |
| Old plants | 3.55 | 3.51 | −1.13 |
| Roots | 1.48 | 1.42 | −4.05 |
| *Lespedeza* | 3.38 | 3.40 | +0.58 |
| Egg mash (1) | 3.07 | 3.065 | −0.16 |
| Egg mash (2) | 3.14 | 3.13 | −0.32 |
| Purina calf chow | 4.25 | 4.31 | +1.41 |
| Dairy ration | 3.23 | 3.13 | −3.09 |
| Mixed feed | 2.23 | 2.38 | +6.72 |
| Cottonseed meal | 6.26 | 6.25 | −0.16 |

TABLE II

TOMATO TISSUE (PERCENTAGE OF GREEN TISSUE BY CHLORATE METHOD)

| SAMPLE | DETERMINATION 1 | DETERMINATION 2 | PERCENTAGE ERROR FROM AVERAGE |
|---|---|---|---|
| | % | % | % |
| Plant 1, stems | 0.1137 | 0.1126 | 0.44 |
| Plant 2, stems | 0.1042 | 0.1000 | 2.01 |
| Plant 1, leaves | 0.3677 | 0.3572 | 1.52 |
| Plant 2, leaves | 0.4167 | 0.4167 | 0 |
| Young lettuce plants | 0.2273 | 0.2232 | 0.88 |

in figuring the percentage of error. Table II presents duplicate results obtained on green tissue samples.

These results indicate that the presence of water does not affect the determination. In fact it is to be expected that water would not interfere, since 50 per cent. sulphuric is used. The interference by water is a serious objection to the Kjeldahl method when used on certain samples, as shown by RANKER (**6**).

The errors from theoretical are not large excepting in the case of pyridine (table III). The stable sulphate formed with pyridine evidently

TABLE III

DETERMINATION OF NITROGEN IN PURE ORGANIC COMPOUNDS

| COMPOUND | THEORETICAL N ADDED | N FOUND | PERCENTAGE ERROR |
|---|---|---|---|
| | *mg.* | *mg.* | % |
| Picric acid | 18.337 | 18.48 | + 0.77 |
| Picric acid | 9.168 | 9.20 | + 0.34 |
| Sulphanilic acid | 6.692 | 6.67 | − 0.33 |
| Sulphanilic acid | 6.692 | 6.54 | − 2.27 |
| Strychnine sulphate | 3.27 | 3.26 | − .33 |
| Pyridine | 8.849 | 2.20 | − 75.25 |

was attacked very little by the oxidizing agent. It will be noted, however, that strychnine gave almost theoretical results. Evidently alkaloids which are derivatives of pyridine or quinoline are readily oxidized if side chains are present, but when no side chain is present to form a path into the ring, the alkaloid is not broken down. The Kjeldahl method is not effective on pyridine or quinoline either.

The low results with sulphanilic acid were probably due to slight impurities since this acid is difficult to obtain absolutely pure.

The nitrogen in a mixture of 50 mg. of pyridine and 50 mg. of young tobacco plant (same sample as in table I) was determined in the usual way. The nitrogen found gave exactly 4.35 per cent. nitrogen for the tobacco, leaving no N for pyridine. Evidently the pyridine was not attacked at all in the presence of the tobacco.

### Part II: Determination of soluble nitrogen

In 1929 a method for the rapid determination of nitrate nitrogen was published (**3**). This method has proved valuable in work with succulent plants, especially of the stem tissues which are rather easy to free of interfering organic substances. The use of copper hydroxide produces satisfactory extracts for the application of the phenoldisulphonic acid method, where the amount of nitrate nitrogen is high in relation to the carbo-

hydrate present, as in succulent, rapidly growing stem tissues of tomatoes, lettuce, cabbage, etc. In leaf tissues and in woody stems, however, interference by carbohydrates and other organic compounds is often encountered. It was found that legumes with zero nitrate grew luxuriantly. Evidently these plants were getting all the nitrogen for growth in a form other than nitrate. There is reason to believe that considerable nitrogen is absorbed from the soil and soil air in forms other than nitrate, especially through the action of root nodules and by fertilization with ammonium sulphate or calcium cyanamid. TIEDJENS and ROBBINS (**7**) and others have shown that ammonia nitrogen is freely assimilated by plants. In order to overcome interference by soluble organic compounds and also to determine the total nitrogen available for metabolism, a method for determining the total nitrogen in plant extracts was devised. This nitrogen, since it includes all nitrogen in true solution in the plant extract, was termed soluble nitrogen. The method consists in oxidizing all material completely by the use of sodium chlorate. This causes all the nitrogen to be converted to nitrate and decomposes all organic compounds, leaving only nitrate and traces of metals, phosphate phosphorus, etc., in solution. The interference by organic material is entirely overcome. Most of the chlorine is driven off; some chloride is present after oxidation, but this does not cause loss of nitrogen as previously explained in note 3 under the total nitrogen determination. Notes 2, 4, and 7 also apply here.

### PROCEDURE

Introduce by means of a graduated pipette 0.1–0.5 cc. of the plant extract depending on the concentration of nitrogen (extraction with weak acetic acid as previously described (**4**) seems preferable), into the bottom of a test tube (20 × 150 mm.) without wetting the sides of the tube. The sample should contain at least 0.005 mg. of nitrogen. The addition of reagents should be in proportion to the size of the aliquot used. Add a granule of sodium chlorate about the size of a grain of wheat. Add 0.2–1.0 cc. of fuming sulphuric acid (15 per cent. $SO_3$) rather rapidly and without shaking so that the solution will boil and excess chloric acid will be decomposed. Shake and blow across the top of the tube until all the chlorine is expelled as indicated by the disappearance of the greenish yellow color and the formation of a perfectly clear, colorless solution. Immediately add 0.1–0.5 cc. of phenoldisulphonic acid and shake. After about 30 seconds add 2.0–10 cc. of water and clear 40 per cent. sodium hydroxide until the maximum yellow color develops and an excess of alkali is present. Care must be taken, if a large aliquot and amount of reagent have been used, to prevent excessive boiling and loss of solution. It may be necessary to transfer to a larger vessel for the neutralization. Make the yellow solution to a volume which brings the color fairly close to the color of the standard

used and compare in a colorimeter. The standard used for results presented here contained 0.0025 mg. of nitrogen per cc. A 1- or 2-gm. sample of green tissue was extracted by grinding with about 0.1 gm. acid-treated charcoal and 10 cc. of 2 per cent. acetic acid.[2]

### Variations in soluble nitrogen

Table IV shows that the method gave very good results on aliquots from the same sample, and that the differences between samples from the same

TABLE IV

Variations in amounts of soluble nitrogen between aliquots of same extract, petioles of same plant, and plants within a few feet of each other on same field plot

| Plant | Aliquot | | Percentage of general average |
|---|---|---|---|
| | 1 p.p.m. | 2 p.p.m. | |
| | Petiole | | % |
| 1 — 1 | 696 | 696 | 96.4 |
| 1 — 2 | 696 | 690 | 96.0 |
| 1 — 3 | 580 | 580 | 80.3 |
| 1 — Average | 656.3 | | 90.9 |
| 2 — 1 | 696 | 696 | 96.4 |
| 2 — 2 | 617 | 617 | 85.4 |
| 2 — Average | 656.5 | | 90.9 |
| 3 — 1 | 867 | 867 | 120.1 |
| 3 — 2 | 906 | 906 | 125.5 |
| 3 — Average | 886.5 | | 122.8 |
| General average | 722.1 | | 100. |

plant probably were due to actual variations in soluble nitrogen in the different petioles taken for analysis. It is possible that the variations were at least partly caused by variations in the extraction process, but it is natural to expect fluctuations in petioles taken from different parts of the plant and which must vary to some extent in age and size. Of course, an even larger variation is to be expected between different plants even if they presumably are in the same soil type.

### Comparisons between soluble and nitrate nitrogen

The question naturally rises as to how much more soluble nitrogen there is than nitrate nitrogen. Table V shows that plants differ widely in the ratio of soluble to nitrate nitrogen although the ratio was quite constant for a particular type of plant in most cases. Lima beans gave an indefinite

[2] Weakened fuming sulphuric acid or isolation of particles of chlorate on the sides of the tube may result in incomplete decomposition of chloric acid. This will act on phenoldisulphonic acid and cause off-color tints.

## TABLE V

COMPARISON OF AMOUNTS OF SOLUBLE NITROGEN TO NITRATE NITROGEN IN SAME PLANT EXTRACT. EFFECT ON PHOSPHATE PHOSPHORUS IN THE EXTRACT (P.P.M. OF GREEN TISSUE)

| SAMPLE | SOIL AND TREATMENT IN LB. PER ACRE | SOLUBLE N | NITRATE N | RATIO SOLUBLE N: NITRATE N | PHOSPHATE P |
|---|---|---|---|---|---|
| | | *p.p.m.* | *p.p.m.* | | *p.p.m.* |
| Tomato (young) lower petioles | Red clay | 4167 | 910 | 4.6 | 320 |
| Tomato (young) lower petioles | Red clay | 4310 | 910 | 4.7 | 266 |
| Tomato (young) lower petioles | Red clay and superphosphate, 500 lb. | 3012 | 1000 | 3.0 | 364 |
| Tomato (young) lower petioles | Red clay and superphosphate, 500 lb. | 3049 | 1062 | 3.0 | ..... |
| Tomato (old) lower petioles | Black loam | 2500 | 625 | 4.0 | 645 |
| Tomato (old) lower petioles | Black loam | 2777 | 625 | 4.4 | 645 |
| Tomato (old) lower petioles | Black loam | 2777 | 555 | 5.0 | 667 |
| Cabbage (half-grown) midribs | Red clay plus $(NH_4)_2SO_4$, 250 lb. | 1661 | 1190 | 1.4 | 80 |
| Cabbage (half-grown) midribs | Red clay plus $CaCN_2$, 250 lb. | 805 | 570 | 1.4 | 100 |
| Cabbage (half-grown) midribs | Red clay plus $NaNO_3$, 250 lb. | 1340 | 925 | 1.4 | 74 |
| Cabbage (half-grown) midribs | Check | 670 | 570 | 1.2 | 143 |
| Peaches growing tips | Black loam | 260 | 55 | 4.7 | 222 |
| Peaches growing tips | Black loam plus $(NH_4)_2SO_4$, 3 lb. per tree | 416 | 0 | ∞ | 92 |
| Lima beans (old plants) main stems | Red clay (check) | 250 | 0 | ∞ | 25 |
| Lima beans (old plants) main stems | Red clay plus superphosphate, 2000 lb. | 261 | 0 | ∞ | 83 |
| Lima beans (old plants) main stems | Red clay plus 8 ton limestone | 160 | 0 | ∞ | 39 |
| Lima beans (old plants) main stems | Black loam (check) | 420 | 0 | ∞ | 125 |
| Lima beans (old plants) main stems | Black loam plus nitrate, 400 lb. | 2500 | 1390 | 1.8 | 31 |

ratio since the nitrate was zero. They evidently were feeding entirely on nitrogen other than nitrate. However, when large amounts of nitrate were added considerable nitrate was present in the stems and caused a ratio of 1.8. Tomatoes maintained a ratio between 3.0 and 5.0, while cabbage was a still more vigorous collector of nitrate, maintaining a ratio of 1.4 even in the presence of ammonium sulphate and calcium cyanamid. The data on peaches indicate that peaches may collect nitrogen in the ammonium form if so supplied.

### Effect of soluble nitrogen on phosphate phosphorus

Except on soil to which superphosphate was added, increase in soluble nitrogen in the plant was almost always associated with a marked decrease in phosphate phosphorus. This is brought out very strikingly in table V. Evidently, when plenty of soluble nitrogen is present, phosphate phosphorus becomes limited because of the stimulus to rapid growth and consequent rapid utilization of the phosphate supply.

### Relation of soluble nitrogen and phosphate phosphorus to plant vigor and yield

Increases in soluble nitrogen correlate with plant vigor but not always with yield. This is shown in table VI, by the lima beans. Medium sized vigorous bean vines on level highland, with a medium soluble nitrogen and relatively high phosphate content, yielded six times as much as large, dark green, vigorous vines in a rich lowland. The content of soluble nitrogen in those in the lowland was three times as large, and that of phosphate was larger than in those on the highland. Despite the higher phosphate, the yield was smaller because the very high soluble nitrogen threw the vines to vegetative growth. In beans the soluble nitrogen-phosphate ratio is one of the most important factors determining yield. Determinations of nitrate in lima beans would not mean much, especially if vigorous nodule formation takes place. The same would probably hold true with peaches as with lima beans. It is especially hard to determine nitrate in peaches since organic substances (probably largely amygdalin) interfere in the test.

Main stems of lima beans, large new shoots of peaches (not the growing tip), and large midribs of cabbage leaves were used for analysis (table VI). Different shoots from the same tree were used in securing the duplicate results on peaches. The yields of cabbage (variety Jersey Wakefield) are pounds for 200 feet of row. The season was rather dry and the heads small.

In cabbage, however, the determination of nitrate is easy and probably is significant, although soluble nitrogen is undoubtedly about as significant. It seems, however, that cabbage is an especially heavy feeder of nitrate and

## TABLE VI

RELATION OF SOLUBLE NITROGEN AND PHOSPHATE PHOSPHORUS TO PLANT VIGOR AND YIELD

| CROP | LOCATION OR TREATMENT | SOLUBLE N | PHOSPHATE P | RATIO N/P | NOTES ON VIGOR AND YIELD |
|---|---|---|---|---|---|
| Lima beans | On sloping land with considerable wash | 143 | 95 | 1.5 | Comparative yields at one picking, 8 lb. (green beans and pods) per 200 ft. of row |
| | On level high land | 298 | 160 | 1.9 | Per 200 ft. of row, 25 lb.; medium sized vigorous green vines |
| | In rich bottom | 893 | 190 | 4.7 | Per 200 ft. of row, 4 lb.; very large over-vegetative dark green vines |
| Peaches | On top of hill (medium slope) | 699 | 571 | 1.2 | Leaves yellowish and dropping off; trees medium size (samples taken July 13) |
| | | 767 | 571 | 1.2 | |
| | At bottom of hill | 1736 | 1111 | 1.6 | Vigorous large trees with dark green leaves, very few leaves dropping |
| | | 1838 | 900 | 2.0 | |
| Cabbage | Check | 670 | 143 | 4.7 | Yield 58 lb.; light green and small |
| | 250 lb. $CaCN_2$ per acre | 805 | 100 | 8.1 | Yield 84 lb.; dark green and large |
| | 250 lb. $NaNO_3$ | 1340 | 74 | 18.1 | Yield 113 lb.; dark green and large |
| | 250 lb. $(NH_4)_2SO_4$ | 1661 | 80 | 20.8 | Yield 71 lb.; medium green and fairly large |

does best when fertilizer is added as nitrate, since sodium nitrate produced the largest yield, despite the fact that ammonium sulphate produced the highest soluble nitrogen content. Previous work (**2**) indicates that an acid soil reaction lowers utilization of nitrogen in the meristem while an alkaline reaction favors its utilization. Since ammonium sulphate tends to produce acidity and sodium nitrate, alkalinity, this may explain why ammonium sulphate caused an accumulation of soluble nitrogen in the cabbage midrib, while sodium nitrate produced larger yields despite the slightly lower soluble nitrogen reserve in the midribs. Anything which slows up nitrogen

utilization is likely to cause an accumulation of soluble nitrogen in the conducting tissues if there is a vigorous nitrogen uptake by the roots. The fact that calcium cyanamide produced a larger yield than ammonium sulphate, despite a lower soluble nitrogen content, gives further proof of the beneficial effect of alkaline fertilizers on cabbage, since cyanamide is much more alkaline than sodium nitrate and probably causes a rapid nitrogen utilization. TIEDJENS and ROBBINS (7) show definitely that ammonia is assimilated as ammonia in an alkaline reaction in sand cultures and that the tomato and soy bean make better growth with ammonium hydroxide than with either nitrate, calcium, or ammonium sulphate. Poor growth was made with ammonium sulphate except when the pH was around 8. Peach, apple, and rose seedlings grew best with ammonia in an alkaline medium. Nitrate was assimilated best in an acid reaction, however, although growth was not so good as with ammonia in an alkaline reaction.

It seems from the data presented in this paper and from previous work on tomatoes, that an extensive study to determine the optimum amounts and ratios of soluble nitrogen and phosphate phosphorus for each type of plant would be of great technical interest and practical importance.

UNIVERSITY OF KENTUCKY
LEXINGTON, KENTUCKY

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# DEVELOPMENT OF THE CHLOROPHYLL AND CAROTENOID PIGMENTS IN BARLEY SEEDLINGS[1]

G. MACKINNEY

## Introduction

This paper reports the results of some experiments showing the effect of modification of the supply of iron and potassium on the formation of the plastid pigments in barley seedlings. In many types of experiments dealing with vital phenomena it is virtually impossible to segregate direct from indirect effects. In the present case, one method would be to show that if other vital processes have not been stimulated so much as pigment formation, then the effect of the element under consideration has probably been direct, and it has a rôle in the mechanism of pigment formation. The difficulty is that the simplest measure of vital activity, increase in dry weight, is itself dependent, although in no simple way, on the chlorophyll itself. While all vital processes may be affected by a particular treatment, the significance of changes in the pigment content is not necessarily vitiated by corresponding growth changes. The writer has attempted a different technique. By withholding iron, plants were allowed to become chlorotic while growing in solutions ample and restricted with respect to potassium. Chlorophyll formation (and therefore growth to some extent) hinged on iron as the limiting factor. Iron was subsequently added to determine whether the previous nutritional treatment had affected pigment formation. In this paper, however, the emphasis will be placed on certain observed pigment interrelationships the ratios of which are unaffected by growth considerations, since it is realized that without extensive studies of potassium nutrition on growth, ranging from definite deficiencies to luxury consumption of this element, with resultant changes in pigment content, this aspect of the question cannot be satisfactorily settled.

## Methods of analysis

The transmission (T) and thence the optical densities (D) of extracted solutions of the various pigments were determined in a spectral region of high absorption for each pigment with a Bausch and Lomb spectrophotometer. The method has been described by MCNICHOLAS (**2**) and SMITH (**9**).

The angle $\theta'$ of rotation of the prism, for a match point, was determined in one field (average of ten readings) and the corresponding $\theta''$ with reversed fields.

Whence $\log T = D = \log \cot \theta' + \log \tan \theta'' = Ech$, where c is the concen-

[1] Contribution from the Division of Plant Nutrition, University of California.

tration of the solution, h the height of the column, and E a constant dependent on the wave length and the nature of the substance.

To obtain E, solutions of known concentration of the pure pigment were prepared and their optical densities determined. At different dilutions, with columns of different height, the constant E could be determined with an accuracy of $\pm 2$ per cent. By means of a vernier, the plunger could be set to 0.1 mm. By using open cups with plungers instead of sealed tubes, and the less volatile solvents, rapid adjustment of the height of the column was possible. In this way intensities at the match point were adjusted for minimum visual strain and greatest sensitivity. The standards were prepared essentially by the method of SCHERTZ (**6**). As several leaf carotenes are optically inactive, the carotenoids were prepared from sunflower leaves. Carrot root carotene is undesirable as it contains 10–20 per cent. of the alpha component.

In unknown solutions, chlorophyll was determined directly in an aliquot of the crude extract. The carotene and xanthophyll were then isolated from the remainder and determined separately, to avoid interference. Comparisons were made for chlorophyll in acetone solution, at 665 mμ; for carotene, in acetone at 500 mμ; and for xanthophyll, in 90 per cent. methanol at 485 mμ.

## Experimental errors

As noted, the constant E was determined with an accuracy of $\pm 2$ per cent. Plant variability was minimized by rigid selection of those seedlings which at the end of a specified time made uniform growth response to constant environmental conditions. Taking the xanthophyll pigment, which showed the greatest variation, in experiment 1, the mean of the xanthophyll in triplicate determinations involving thirty plants is 1.08 mg. $\pm$ 0.04 per ten plants. We may estimate therefore the gross experimental error to be not greater than $\pm 5$ per cent. It must be pointed out that when an element such as iron is present in limiting amounts, control sets do not represent duplicates since minute traces of iron both in the plant and as impurity in the solution will affect the pigment content. Equally rigid exclusion of iron is impossible in cultures of this type, nor is this necessary beyond the point where the plant becomes sensitive to the low concentration of iron.

## Experimentation

With variations in the pigment content under various treatments there will be variations in respirable carbohydrates, and consequently in absorption of minerals from the culture solution. In certain experiments these differences have been minimized by subjecting the plant to a given treatment for a period of time, then transferring it to dilute calcium sulphate

solution for observation. In so far as pigment formation is determined by mineral nutrition, changes can occur only to the extent that the plant has stored up the necessary minerals. This necessitates experiments of relatively short duration.

### Experiment I: Pigment ratios in normal and chlorotic barley seedlings

Barley seedlings of height 6 cm. ± 0.5, seven days after germination at 20° C., were transferred to twenty 1-quart jars (six plants per jar) containing Hoagland's solution, with supplementary constituents[2] except for iron. Ten of these jars received 5 mg. Fe from a 1 per cent. solution of ferrous sulphate per week. Iron was excluded from the remainder. Plants from cultures with and without iron were harvested periodically in duplicate sets. The results are shown in table I. Agreement is to be anticipated for

TABLE I

Pigments in normal and chlorotic barley plants (mg. per 10 plants)

| | Chlorophyll | Carotene | $Q\frac{a+b}{c}$ | Xantho-phyll | $Q\frac{c}{x}$ |
|---|---|---|---|---|---|
| | *mg.* | *mg.* | | *mg.* | |
| Initial | 1.03 | 0.0336 | 30.7 | 0.0948 | 0.35 |
| | Normal series (iron added) | | | | |
| 8 days | 6.54 | 0.222 | 29.5 | 0.508 | 0.44 |
| 10 days (1) | 9.70 | 0.32 | 30.3 | 0.594 | 0.54 |
| (2) | 9.38 | 0.312 | 30.0 | 0.474 | 0.66 |
| Outer leaf | 2.62 | 0.085 | 30.8 | 0.118 | 0.72 |
| remainder | 7.18 | 0.254 | 28.3 | 0.428 | 0.59 |
| Total (3) | 9.80 | 0.339 | 28.8 | 0.546 | 0.62 |
| 12 days | 12.82 | 0.412 | 31.1 | 0.744 | 0.55 |
| 19 days | 21.4 | 0.712 | 30.0 | 1.15 | 0.62 |
| | Chlorotic series (iron restricted) | | | | |
| 8 days | 4.72 | 0.16 | 29.5 | 0.470 | 0.34 |
| 10 days (1) | 3.12 | 0.110 | 28.4 | 0.346 | 0.32 |
| (2) | 4.06 | 0.156 | 26.1 | 0.370 | 0.42 |
| Outer leaf | 2.22 | 0.076 | 29.2 | 0.136 | 0.56 |
| remainder | 3.26 | 0.122 | 26.7 | 0.324 | 0.38 |
| Total (3) | 5.48 | 0.198 | 27.7 | 0.460 | 0.43 |
| 12 days (1) | 3.08 | 0.100 | 30.8 | 0.238 | 0.42 |
| (2) | 4.16 | 0.134 | 31.0 | 0.294 | 0.46 |
| 19 days | 2.76 | 0.079 | 34.7 | 0.216 | 0.37 |

duplicate sets receiving iron; but, as already noted, each set of chlorotic plants will not have had equally rigid exclusions of this element, causing greater variation. In the outside leaves of plants without iron the color was apparently normal, whereas the inside leaves were severely chlorotic.

[2] A–Z constituents, *cf.* Schropp and Scharrer (7).

After a 10-day period, from three jars of each series the outside leaves were sampled for comparison with those inside. The results of the experiment show:

1. The chlorophyll: carotene ratio, $Q(a+b)/c$, is remarkably constant. One infers a straight line relationship of the type $y = mx$ between the two pigments, in barley seedlings, whether they are developing normally or becoming chlorotic. The relationship between chlorophyll and carotene has been observed by EULER (1) and SJØBERG (8) in etiolated barley seedlings exposed to light, and more generally between chlorophyll and total carotenoids by OSERKOWSKY (5) in normal and chlorotic pear leaves.

2. The carotene: xanthophyll ratio, Q c/x, with normally developing seedlings rapidly approaches the value of about 0.60, determined by WILLSTÄTTER. The Q c/x for chlorotic plants is consistently lower, with one interesting exception, the green outside leaves of plants that are becoming chlorotic.

### EXPERIMENT II: PIGMENT FORMATION AS AFFECTED BY HIGH AND LOW CONCENTRATIONS OF POTASSIUM IN THE EXTERNAL SOLUTION

The potassium ion is readily absorbable, and modification of its concentration causes marked effects on the accumulation of $Ca^{++}$, $Mg^{++}$, and $(NO_3)^-$ ions. Analyses are given in table II showing that the presence or absence of iron has little effect on potassium withdrawals by the plant. However, nothing is known of the effect of potassium on the absorption of supplementary constituents, such as boron or copper, which are toxic in all but high dilutions although essential for normal growth. It may not be justifiable to assume that the amount of copper required by the plant is so small that a tenfold variation in the $K^+$ concentration does not, under some conditions, inhibit to a limiting degree the absorption of copper. In experiments II and III, a modified Hoagland solution was used, high $K^+$ series receiving 200 mg. and low $K^+$ series 20 mg. of potassium per liter. The latter received the corresponding sodium salts to maintain the total salt concentration.

TABLE II

ABSORPTION OF POTASSIUM

| SERIES | K IN SOLUTION | | ABSORBED BY PLANT |
|---|---|---|---|
| | INITIAL | FINAL | |
| | *mg.* | *mg.* | *mg.* |
| 1. High K | | | |
| (a) + Fe | 200 | 72.2 | 127.8 |
| (b) − Fe | 200 | 67.7 | 132.3 |
| 2. Low K | | | |
| (a) + Fe | 20 | 1.5 | 18.5 |
| (b) − Fe | 20 | 1.0 | 19.0 |

Seeds were germinated and selected as before. Thirty-six quart jars were set out, eighteen receiving the high $K^+$ solution and eighteen the low $K^+$.

Six of each series were given 5 mg. Fe, as before. The plants were allowed to grow for 10 days in their respective culture solutions. Samples of each treatment from both series were then taken in duplicate. The

TABLE III

PIGMENT CONTENT AS AFFECTED BY IRON AND POTASSIUM MODIFICATIONS (MG. PIGMENT IN 10 PLANTS)

| | CHLOROPHYLL | CAROTENE | $Q\frac{a+b}{c}$ | XANTHOPHYLL | $Q\frac{c}{x}$ |
|---|---|---|---|---|---|
| | *mg.* | *mg.* | | *mg.* | |
| Initial | 0.740 | 0.024 | 30.8 | 0.054 | 0.44 |
| A*–1 | 6.28 | 0.160 | 39.3 | 0.330 | 0.48 |
| | ..... | 0.126 | ..... | 0.257 | 0.49 |
| B–1 | 5.92 | 0.163 | 36.3 | 0.278 | 0.59 |
| | 5.52 | 0.166 | 33.3 | 0.316 | 0.53 |
| A–2 | 3.80 | | | | |
| | 3.00 | 0.096 | 31.3 | 0.307 | 0.31 |
| B–2 | 4.18 | 0.104 | 40.1 | 0.248 | 0.42 |
| | 3.63 | 0.100 | 36.3 | 0.210 | 0.48 |
| | Plants were here transferred to $CaSO_4$ solution: After 4-day period | | | | |
| A–1 | 8.07 | 0.237 | 34.1 | 0.467 | 0.51 |
| B–1 | 5.12 | 0.142 | 36.1 | 0.288 | 0.49 |
| A–2a | 6.63 | 0.162 | 40.9 | 0.327 | 0.50 |
| | 6.48 | 0.151 | 42.9 | 0.327 | 0.46 |
| B–2a | 4.75 | 0.131 | 36.3 | 0.223 | 0.59 |
| | 4.55 | 0.129 | 35.3 | 0.220 | 0.59 |
| A–2 | 3.98 | 0.106 | 37.5 | 0.242 | 0.44 |
| B–2 | 3.42 | 0.090 | 38.0 | 0.176 | 0.51 |
| | After 7-day period | | | | |
| A–2a | 6.24 | 0.188 | 33.2 | 0.391 | 0.48 |
| | 6.03 | 0.192 | 31.4 | 0.342 | 0.56 |
| B–2a | 4.05 | 0.123 | 32.9 | 0.218 | 0.56 |
| | 4.23 | ..... | ..... | 0.281 | ..... |

* A denotes high K; B, low K solutions.
1 " 5 mg. Fe from beginning and 5 mg. at transference to $CaSO_4$ solution.
2 " no Fe.
2a " Fe from time of transference to the $CaSO_4$ solution.

solutions in the remaining jars were then discarded and replaced by dilute calcium sulphate solution. Each series which had not received iron was subdivided: half of them were given 5 mg. Fe and iron was again withheld from the remainder. This was done to determine whether the plants, in recovering from the chlorotic conditions which had developed, would show pigment differences ascribable to their previous nutritional treatment. The

plants were allowed to develop in the dilute calcium sulphate solution, and were sampled in duplicate after four and seven days. No further samples were taken as the plants began to show the effect of restrictions in the nutrient solutions. In table III are given the pigment analyses on the basis of ten plants. It may be noted that, except for color, the plant tops showed no visible differences in development, although roots were whiter and possibly less sturdy in the absence of iron. As already pointed out, however, there is no appreciable difference in the respective potassium withdrawals, so that one may assume that there is no marked difference in the capacities of the root systems for absorption of mineral nutrients.

After the 10-day period in the original solution, the plants without iron have become definitely chlorotic, the high $K^+$ series approximately as severely as the low $K^+$. Iron has therefore been the limiting factor in chlorophyll formation, and potassium has not been able to act as a substitute. After the plants have been transferred to the calcium sulphate solution for four days, the chlorophyll content of plants in the high $K^+$ series is nearly doubled, where iron has been added, while there is less than a 20 per cent. increase in the corresponding plants of the low $K^+$ series. Where iron has been withheld, no pigment increase can be noted in either series. It will be seen that while there is a fairly definite relationship between the chlorophyll and the carotene, the magnitude of $Q(a+b)/c$ is not strictly comparable with that of experiment I, which was confined to two phases: (1) normal development in seedlings, and (2) arrested development due to incipient iron chlorosis. In this experiment the leaves are recovering from chlorosis under varying nutritional conditions. The ratio $Q\,c/x$ shows a less marked lowering for the low $K^+$ series than for the high $K^+$. The general fluctuation of chlorophyll with the carotenoids is noticeable throughout.

### Experiment III: Effect of variation in potassium content on pigment formation in the dark

Trays of barley seedlings were germinated at 20° C. in the dark over the solutions high and low with respect to the $K^+$ concentration. The condition of the plants after 12 days is shown in table IV. Duplicate samples of fifty plants each from both series were taken after 6, 9, 11, and 15 days. Results of pigment analyses are shown in table V. No chlorophyll was detectable. An apparent trace was measurable spectrophotometrically at 665 mμ. But when comparisons were made also at 670 mμ and 660 mμ, the same divergence (less than 0.5°) was noticeable in the setting of the prisms for a match point. Obviously no definite absorption band of chlorophyll was demonstrable. It is possibly not to be expected that seedlings steadily losing weight in the dark should show, in their pigment content, definite

TABLE IV

COMPOSITION OF PLANTS

| SERIES | DRY WEIGHT OF 50 PLANTS | K ON DRY WEIGHT BASIS |
|---|---|---|
| | *gm.* | % |
| High K | 1.42 | 2.44 |
| | | 2.37 Average 2.405 |
| Low K | 1.66 | 0.47 |
| | | 0.61 Average 0.54 |

TABLE V

PIGMENT IN PLANTS GROWN IN THE DARK (MG. PER 50 PLANTS)

| | XANTHOPHYLL | | CAROTENE | | $Q\frac{c}{x}$ |
|---|---|---|---|---|---|
| | HIGH K | LOW K | LOW K | HIGH K | |
| | *mg.* | *mg.* | *mg.* | *mg.* | |
| 6 days (1) | 0.046 | 0.034 | 0.003 | 0.002 | |
| (2) | 0.080 | 0.054 | 0.005 | 0.005 | |
| Average | 0.054 | | 0.004 | | 0.07 |
| 9 days (1) | 0.111 | 0.130 | 0.011 | 0.020 | |
| (2) | 0.102 | 0.120 | 0.008 | 0.014 | |
| Average | 0.115 | | 0.013 | | 0.11 |
| 11 days (1) | 0.107 | 0.148 | 0.008 | 0.015 | |
| (2) | 0.173 | 0.122 | 0.008 | 0.017 | |
| Average | 0.137 | | 0.014 | | 0.10 |
| 15 days (1) | 0.156 | 0.144 | 0.017 | 0.017 | |
| (2) | 0.178 | 0.174 | 0.021 | 0.020 | |
| Average | 0.163 | | 0.019 | | 0.12 |

response to the solutions. The low values of Q c/x confirm the findings of EULER (**1**) and SJØBERG (**8**).

An apparent conflict may be noted between the results of these two workers. The former notes that where carotene is present, it is accompanied by chlorophyll, and its production is ascribed to incomplete darkness. The latter invariably finds carotene, and rarely chlorophyll. Superficially, the writer's results substantiate the latter viewpoint. But conclusions that light may not be necessary for carotene formation in photosynthetic tissue, or that chlorophyll is not necessary for carotene production, are by no means valid. Vital processes in the etiolated seedling have been seriously interfered with. Reactions proceeding in the normal plant have been profoundly modified, and the direction of some may have been reversed. In this paper the writer shows the interdependence of chlorophyll and carotene under certain conditions. This is not true for all conditions. Thus, in green tissue placed in the dark the ratio $Q(a+b)/c$ falls, while $Q\,c/x$ rises. Therefore the reactions which have brought about traces of carotenoid pigments in the etiolated seedlings may not be those which

are responsible for pigment production in the illuminated plant. In fact there is no evidence as yet of the chemical identity of either carotenoid occurring in the etiolated plant with those of normal green tissue. To obtain production of xanthophyll for example, one must not interfere with the processes which produce chlorophyll. The only valid statement permissible is that the present technique and equipment permitted the measurement of traces of carotene where chlorophyll was not present in measurable amounts.

## Summary

1. Fluctuations are reported in the chlorophyll and carotenoid contents of barley seedlings grown for short periods of time in culture solutions modified with respect to iron and potassium.

2. The constancy of the chlorophyll: carotene ratio noted by EULER and HELLSTRÖM for etiolated seedlings is extended to cover chlorotic seedlings and also those recovering from chlorosis induced by lack of iron.

3. Chlorophyll and carotenoids fluctuate together, in general, although the carotene: xanthophyll ratio is markedly lower in chlorotic leaves.

4 The effect of potassium, whether specific or one of general nutrition, under certain conditions is to increase the pigment content of the plants.

Acknowledgment is made to Professor D. R. HOAGLAND, Division of Plant Nutrition, University of California, for advice and guidance.

ADDENDUM.—In the light of recent developments in methods, a few comments appear pertinent, to attempt to assay more accurately the value of the data presented.

In this work, the greatest fluctuation occurred in the xanthophyll fraction. It has been noted by STRAIN (10) for a number of plants, including barley, that the leaf xanthophyll fraction contains several components, while β carotene is probably the sole constituent of the carotene fraction of this plant. It is evident therefore that a measure of uncertainty is introduced, as any assumption that there has been no change in the proportions of the various components of "total" xanthophyll may be justified only under a limited set of conditions.

Exposing etiolated sunflower seedlings, germinated at 85° F., to light, NORRIS (4) finds a close relationship between chlorophyll and xanthophyll, but none between these pigments and carotene, contrary to the work of EULER and HELLSTRÖM (1) with etiolated barley seedlings. NORRIS utilized a spectrophotoelectric method, isolating the 4358 Å. mercury line for analyses of the two carotenoid fractions. In the experience of MILLER, MACKINNEY, and ZSCHEILE (3) the absorption curves for various carotenoids show greatest reproducibility where the curves are flatter, at the maxima.

For visual work, unless one is matching the intensities of two semicircles in a circular field, it is impracticable to use narrow exit slits. With the Bausch and Lomb spectrometer no. 2700 the writer found it convenient to isolate a spectral region of approximately 6 mμ in magnitude, for chlorophyll (*i.e.*, 668–662 mμ) and from 2.5 to 3.0 mμ for the other pigments. These were not varied for standards and unknowns. Subsequent observations on crude carotenoid extracts, by visual and spectrophotoelectric methods, and to a limited extent by photographic means, lead the writer to believe that while the positions of maxima and minima are identical on the wave-length scale, within the experimental error, with those of the crystalline carotenoid in the same solvent, the gen-

eral shape of the curve is different, the bands being more diffuse and less clearly defined. This might be ascribed to unavoidable traces of a colored and partially oxidized form of the carotenoid.

If this observation be confirmed, there are obvious inherent errors which may affect absolute though not comparative results for methods dependent on a single "wave length" for the determination, especially if that wave length is not in a region of maximum absorption for the pure pigment.

As pointed out, the chlorophyll: carotene relationship found for one set of conditions does not exist for all conditions. The divergent results in the literature emphasize the necessity of critical examination of the method to be used, and the urgency of preliminary studies, by adsorption methods, of the carotenoid complexes which may exist in the plant chosen for study, before satisfactory conclusions can be drawn as to the functions of these pigments in the living leaf.

CARNEGIE INSTITUTION OF WASHINGTON
STANFORD UNIVERSITY, CALIFORNIA

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# ABSORPTION SPECTRA OF ALPHA AND BETA CAROTENES AND LYCOPENE[1]

ELMER S. MILLER, G. MACKINNEY,[2] AND F. P. ZSCHEILE, JR.

(WITH THREE FIGURES)

This paper presents a detailed study of the specific absorption coefficients of alpha and beta carotenes purified by resolution on magnesia, according to the method of STRAIN (**5**), and on calcium hydroxide according to the method of MILLER (**3**), and of lycopene. The absorption coefficients were measured by an improved spectro-photoelectric method which will be described in due course.

From the viewpoint of carotenoid spectroscopy, several important changes have been made. The period required for a single reading has been reduced from 60 to 10 seconds. The length of the liquid path in the absorption cells is 2 cm. ± 0.15 per cent. The Hilger (type D, no. H291) absorption cells have been placed behind slit number II of the monochromator, where they are not subjected to the full intensity of the illuminating source, in this case, a 900-watt Mazda lamp.

In order that comparisons with values obtained in other laboratories may be valid, we deem it essential to state: (1) slit widths in mm. actually employed at the various wave lengths, and (2) the effective dispersion, *i.e.*, the slit width in terms of Å. U. per mm. of slit. From these data the spectral range isolated may be calculated. These calibration data of the optical system must be obtained from constants determined by means of a steady source of monochromatic light of suitable wave lengths. A mercury arc was used in this case.

In this paper are reported values of the specific absorption coefficients for alpha and beta carotenes and lycopene in the wave length range 3900–5300 Å. U. The solvent was 20 per cent. diethyl ether and 80 per cent. absolute ethanol (by volume). 1.5–3.0-mg. samples were weighed on a Kuhlmann microbalance, with a maximum error of approximately ± 0.3 per cent. The samples were dissolved in ether and made up to volume with alcohol. Concentrations employed were approximately 0.6–1.0 mg. per liter. Using fused quartz cells with these concentrations, the light transmission at the maxima for alpha and beta carotenes was from 20–40 per cent. On different preparations, from various sources, values of $a^3$ at the

[1] Contribution from the George Herbert Jones Chemical Laboratory, University of Chicago.

[2] National Research Council Fellow. This investigation employed apparatus made available by a grant from the Rockefeller Foundation, New York, to the University of Chicago.

[3] From the formula $\log \frac{I_x}{I_0} = a\ c\ x$, where $a$ is in liters per gram cm.

maxima (and between 4350 and 4800 Å. U.) were reproducible within ± 0.6 per cent. (total error). For the steeper portions of the curves (on either side of the spectral range between 4350–4800 Å. U.) the total error is ± 1.0 per cent. Absorption coefficients on different samples of the same preparation show that the magnitude of the instrumental and weighing errors contribute to the total error approximately one-half that stated above.

The following slit widths were employed: 0.04–0.01 mm. for wave lengths between 3900 and 4300 Å. U., and 0.01–0.007 mm. between 4300 and 5300

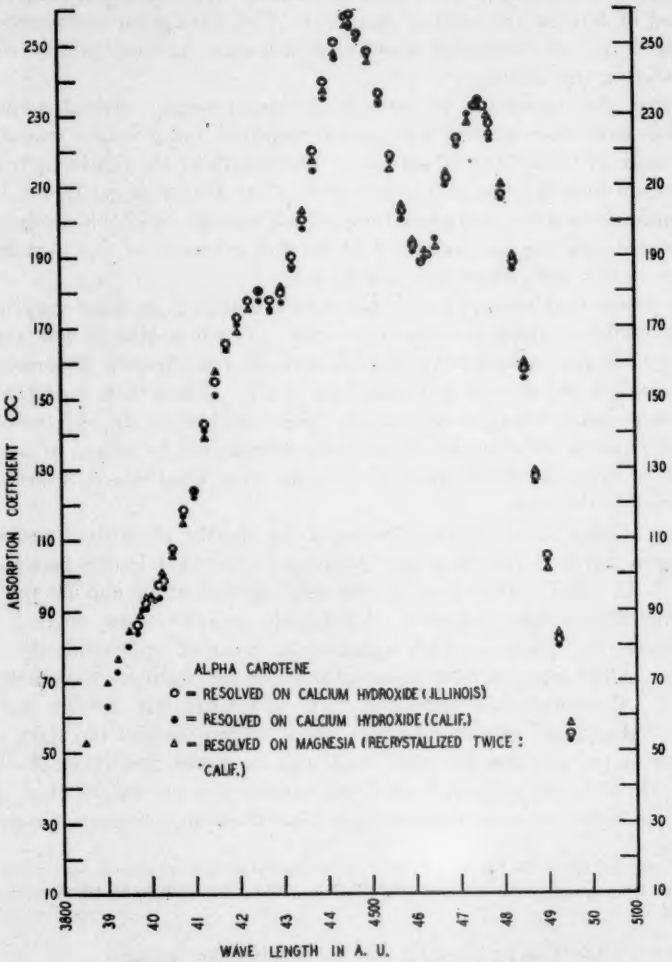


FIG. 1. Absorption spectrum of alpha carotene.

Å. U. These slit widths in terms of Å. U. are 2.5–1.0 and 1.0–1.5 Å. U. respectively. The spectral regions isolated at wave lengths 3900, 4300, and 5300 Å. U. were 7.5, 3.0, and 4.5 Å. U. respectively. When these slit widths were employed, no change was observed in the value of the specific absorption coefficients when a mercury arc was substituted for the Mazda lamp. (Beta carotene and the mercury lines at 3906, 4047, 4078, 4358, and 4916 Å. U. were employed). In order that workers in different laboratories may

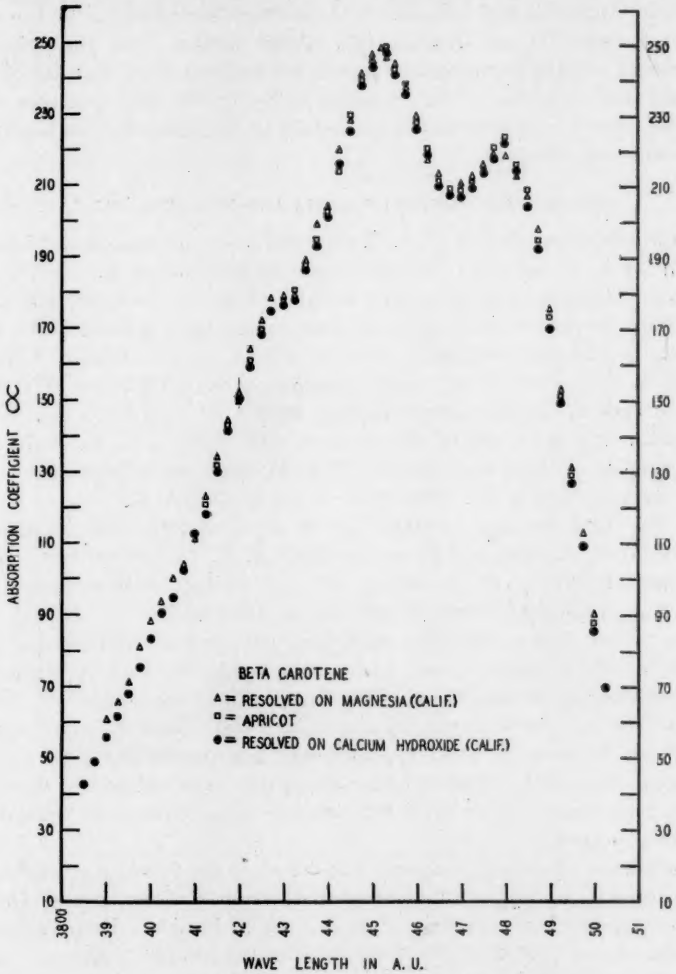


FIG. 2. Absorption spectrum of beta carotene.

compare values, it is essential that data concerning slit widths in terms of mm. and Å. U. per mm., and the spectral limits of the light employed be included with the values of the absorption coefficients.

## Experimentation

### Preparations and purification of alpha and beta carotenes

The sources of the crude alpha and beta carotenes were carrot roots grown in California and Illinois. The carotenes were purified by the methods of Strain (5) and Miller (3). After elution from the respective adsorbents, all the preparations were recrystallized from ligroin[4] rich in heptane and dried for 10 to 14 hours at $2\text{–}40 \times 10^{-5}$ mm. pressure. The samples were recrystallized until a constant set of values for the absorption coefficients was obtained.

### Absorption spectra of alpha and beta carotenes

Readings were taken at 10 Å. U. intervals over the maxima and minima, and at 25 Å. U. intervals for the remaining portions of the curve. The maxima and minima can be located within $\pm$ 5 Å. U. In figure 1 is shown the absorption spectrum of alpha carotene, consisting of 3 bands and a shelf. Maxima for the two main bands occur at 4750 Å. U. ($a = 231.5 \pm 1.5$), and at 4460 Å. U. ($a = 257 \pm 1.5$), with a minimum at 4620 Å. U. ($a = 191.0 \pm 1$). A third band is found at approximately 4250 Å. U. ($a = 178 \pm 2$). While we consistently get a dip in the curve at 4260–4270 Å. U., the differences in this region at three wave lengths 10 Å. U. apart are approximately that of the experimental error. The shelf occurs at 4010 Å. U.

In the curve for beta carotene (fig. 2) are found two definite maxima, at 4800 Å. U. ($a = 222 \pm 1.2$) and at 4525 Å. U. ($a = 249 \pm 1.5$), with a minimum at 4700 Å. U. ($a = 210 \pm 1.2$). A distinct shelf occurs at 4300 Å. U. and an apparent point of inflection at 4050 Å. U.

The values of the absorption coefficients are approximately 15 per cent. higher for alpha carotene, and 4 per cent. higher for beta carotene than those reported by Miller (4) showing that previous results are low, though in shape the two sets of curves are almost identical. This difference may in part be ascribed to a slit width approximately one-quarter the width of those previously used, and in part to better absorption cells. However, these two factors cannot account for the lower value of alpha carotene in comparison with beta carotene.

The curves presented in figures 1 and 2 show the identity of alpha and beta carotenes isolated from carrot roots from California and Illinois whether separated on magnesia or on calcium hydroxide. Preparations of

[4] Using ligroin (B.P. 30–60° C.) it was found desirable to add 10 per cent. heptane to give satisfactory resolution.

beta carotene from the leaves of mint, dandelion, and apricot, isolated by MACKINNEY (1), gave on recrystallization curves identical with those reported in figure 2. A comparison of the curves (figs. 1 and 2) shows the values of the specific absorption coefficients for alpha carotene to be slightly higher than those for beta carotene at their respective maxima.

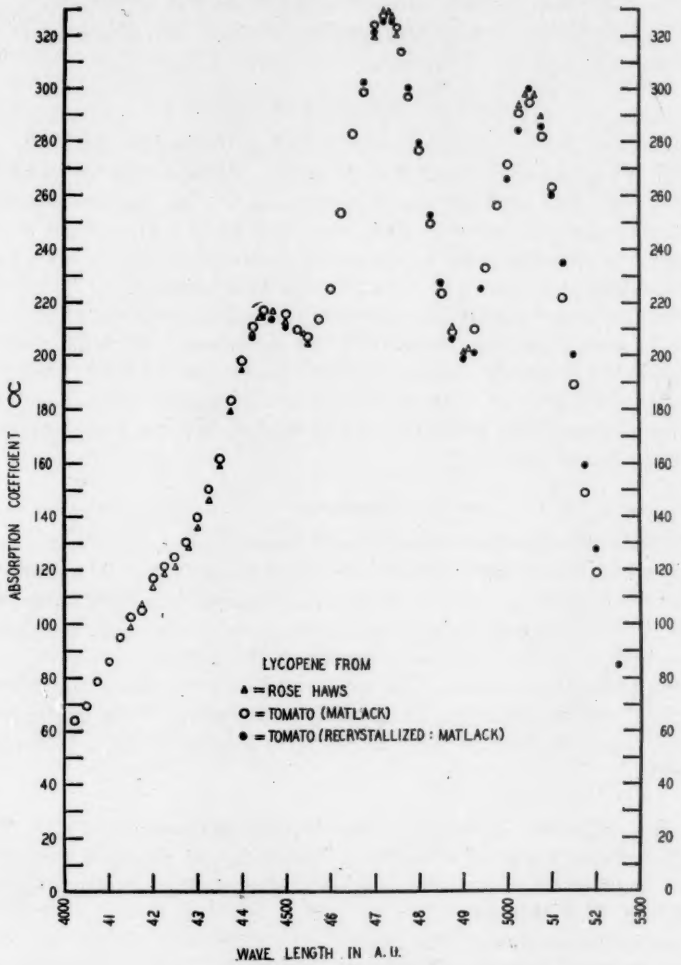


FIG. 3. Absorption spectrum of lycopene.

### Lycopene

Lycopene was obtained from two sources, one isolated by Mackinney (1) from the hips of the California wild rose, the other from tomatoes, furnished by courtesy of M. B. Matlack (2). The rose hips lycopene was isolated from accompanying carotenoids by passage of the petroleum ether crude extract over magnesia according to the method of Strain (5). A check was thus provided on the possible effect of the adsorbent on this pigment.

### Absorption spectrum of lycopene

Three well defined maxima were found at 5050 ($a = 298 \pm 2$), 4725 ($a = 327 \pm 2$), and 4465 ($a = 217 \pm 1$) Å. U. Minima were found at 4900 ($a = 201 \pm 2$) and at 4550 ($a = 204 \pm 2$) Å. U. An apparent point of inflection was noted between 4200 and 4250 Å. U. The values for the absorption coefficients at the maxima were determined with the same degree of precision as that described for alpha and beta carotene.

A less rigorous examination was made of the other portions of the curve. Owing to their steepness, we estimate an uncertainty of $\pm 2.0$ per cent.

Within the relatively narrow concentrations employed here (0.6–1.0 mg. per liter) we have not observed significant deviations from Beer's law. For the most accurate work, between 20 and 40 per cent. of the incident light must be transmitted.

## Summary

Curves of the specific absorption coefficients for alpha and beta carotene and lycopene are presented for the wave length range from 3900 to 5300 Å. U. The carotenes from two different localities resolved into their components on two different adsorbents, magnesia and calcium hydroxide, yield identical products. A discussion is presented of the desirability, in all future work, of stating the magnitude of error and certain fundamental details of the optical system employed, particularly with regard to the purity of the spectral region isolated at the various wave lengths where measurements are made.

Acknowledgment is made of the helpful suggestions of Dr. T. R. Hogness, Department of Chemistry, University of Chicago, and of the cordial cooperation of members of the Division of Plant Biology, Carnegie Institution of Washington.

Carnegie Institution of Washington
Stanford University, California
University of Chicago
Chicago, Illinois

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## CHEMICAL ANALYSIS OF PLANT TISSUE[1]

The present observations and recommendations supplement those previously issued by this committee (**3**). Since that period the subject has received increasing attention by investigators, as becomes apparent in the character of numerous published articles. Appreciative acknowledgment is here made of aid received from a number of persons in the development of this supplement.

It is noteworthy that an extensive treatise for use in research has appeared recently under the direction of KLEIN (**9**). Increasing aid is also found in laboratory manuals of biochemistry, as for example the work of COLE (**2**).

SAMPLING.—In the case of fruits the number of individuals required for a representative sample received attention some time ago by DENNY (**4a**). He found with apples, for example, that 50 units give significance to a difference of 5 per cent. in acidity. The necessity for determination of absolute amounts of a given constituent per plant unit in following its appearance or disappearance with metabolic changes has been demonstrated by KERTESZ (**8a**). By the treatment of peas in groups of equal numbers he demonstrated the fallacy of a common belief that sucrose is converted to starch when canning of the harvested crop is delayed.

PRESERVATION.—In view of information recently made available it appears desirable to qualify the application of preservative treatments to either tissue or extracts. It may thus be possible to aid the individual worker in the selection of a method adapted to his conditions.

*A. Alcoholic extraction.*—Apparently extracts obtained by the use of boiling 80 per cent. alcohol are stable but a few days at room temperatures, at least as regards some of the nitrogenous constituents. WEBSTER (**13**) has reported the occurrence of appreciable deaminization soon after cooling such extracts. It is recommended that changes of composition in stored extracts be retarded by holding at low temperatures, preferably below 0° C.

*B. Freezing.*—Enzyme action is not prevented by the freezing of plant tissues. On the contrary, ruptures caused by this treatment seem to bring enzymes and substrates into contact. For this reason, heat treatment should precede freezing when the samples are to be stored for more than a few weeks. Frozen samples should be wrapped or sealed air tight to prevent dehydration from evaporation, which proceeds rapidly in frozen tissue at −18° C. The temperature of the storage room should be at most −18° C.,

[1] First supplement to Recommendations of the Committee on Methods of Analysis for the American Society of Plant Physiologists. This report was assembled by W. E. TOTTINGHAM for the committee on chemical methods: Z. I. KERTESZ, W. E. LOOMIS, T. G. PHILLIPS, W. E. TOTTINGHAM, chairman.

but it is preferable to operate at $-40°$, where as yet enzyme action has not been shown to have measurable velocity. On enzyme action at low temperature see the review by HEPBURN (6); and the following papers: PHILLIS and MASON (10), JOSLYN and SHERRILL (7), and KERTESZ (8b). Extreme care should be exercised when samples frozen without heat treatment are thawed: they should be defrosted in alcohol, when extractions are to be performed, or heated to inactive enzymes immediately after defrosting to minimize effects upon the composition. Heat treatment and drying, and preservation in alcohol after heating are preferable to preservation by freezing.

*C. Heat drying.*—This method was outlined in our original recommendations. Small samples may be dried quickly at 60° C. *in vacuo,* but it must be recognized that enzymes are not thus inactivated. The Committee recommends, with particular reference to larger samples, prompt inactivation of enzymes by heating to 80° C. for 10 min., followed by drying at 70° in a well ventilated oven. This implies, of course, assurance that the innermost cells of the tissue acquire the specified temperature. Dried tissues should be stored in sealed containers to prevent absorption of water, but this applies more particularly when enzymes have not been inactivated.

EXTRACTION.—The use of alcohol to give at least 80 per cent. strength, and with heating, is to be preferred as an extractive method where one is concerned primarily with the determination of sugars. Without heating, as in the use of a Soxhlet extractor, solubility is greatly diminished. The apprehension expressed earlier regarding possible hydrolysis of sucrose by tissue acids was apparently unjustified. DENNY (4b) found the use of $CaCO_3$ to prevent this action generally unnecessary.

Owing to their lesser solubility, it may be expected that amino acids and other simpler nitrogenous constituents will require more extended extraction than the sugar fraction. For this type of extraction excessive heating of the extract should be avoided. It is well to use successive smaller portions of solvent, limiting the temperature to 80° and its effective time to 10 min. This treatment should largely inactivate enzymes, while hastening the precipitation of colloids.

Increasing yields of extractives as reported by users of the autoclave should be viewed reservedly until the extent of alteration by hydrolysis or otherwise is known. The use of cytolyzing agents in preparation of fresh tissue when it is desired to obtain undiluted sap tends, of course, to release enzymes and calls for speed and low temperatures in the operation. This same risk attends grinding of the tissue with water at ordinary temperatures, but the high solvent power of water must be considered an important asset. STUART (private communication from the University of Maryland) as an average for 9 species extracted 40 per cent. more non-protein nitrogen

with cold water than with hot alcohol. On the other hand, LOOMIS (private communication from Iowa State College) reports a distinct end-point in extraction by alcohol for a considerable variety of tissue samples. It may prove feasible to increase the solvent efficiency of alcohol by dilution to 50 per cent., while retaining to a large degree its stabilizing capacity. To avoid appreciable changes of composition in the treatment of large samples one may adopt extraction by hot water, in the manner practiced by VICKERY and PUCHER (**12**) on tobacco leaves. This procedure combines prompt inactivation of enzyme with high solvent efficiency.

DEFOAMERS AND DEEMULSIFIERS.—Some extracts, more particularly those obtained by pressure only or the use of cold water, tend to froth freely. This property disturbs the establishing of volumes, aeration procedure, and the Van Slyke determination of amino nitrogen. A few drops of ether cut the foam for making to volume, but this agent is lost, of course, in aeration. SCHLENKER (**11**) recommends for aspiration procedure the addition of paraffin dissolved in xylene. Heavy alcohols serve the same purpose but their efficiency is gradually lost. Primary caprylic alcohol is used in the Van Slyke determination, although the secondary form, also designated as methyl-n-hexyl carbinol, seems to be preferred. Attention may be called to directions (**5**) by which secondary caprylic alcohol can be prepared readily from castor oil. H. A. CONNER (private communication, Department of Plant Pathology, University of Wisconsin) recently discovered that a solution of castor oil in ethyl alcohol provides a very efficient foam breaker. Although but a few drops are employed, these agents should be examined as possible sources of a high blank in the microdetermination of alpha-amino nitrogen.

It has been customary to depend upon the water attracting power of certain salts for the purpose of breaking troublesome emulsions. When the presence of nitrates prohibited the introduction of chlorides PHILLIPS (private communication from University of New Hampshire) found it possible to substitute 0.3 per cent. $CaCl_2$ by the molecular equivalent of $MgSO_4$. CHANNON and FOSTER (**1**) overcame persistent emulsions in washing ethereal solutions of lipides by introducing acetone with NaCl (100 parts water, 40 parts acetone, 5 parts salt).

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## DETERMINATION OF CARBOHYDRATES[1]

This report is supplementary to the previous reports of the committee on soluble carbohydrates and polysaccharides (Plant Physiol. **2**: 91–97; 195–204. 1927), and should be used in conjunction with the original reports.

CLEARING METHODS.—The first report recommended clearing sugar solutions with saturated neutral lead acetate solution and deleading with potassium oxalate. MCGILLIVRAY (**10**) finds that clearing does not affect the reducing power of tomato extracts and consequently can be omitted with this material. Undoubtedly clearing of many other materials is more a matter of convenience in handling than of necessity. Whenever sugar solutions do not give a lowered reducing value upon clearing with neutral lead acetate, and can be conveniently handled without clearing, this operation may be considered to be dispensable. Clearing is necessary (a) whenever tannins or similar reducing impurities are present in the extract, and (b) whenever dextrin or inulin extracts (containing, as they ordinarily do, gums and soluble hemicelluloses) are to be hydrolyzed with acid. Note that the clearing is given before the acid hydrolysis.

LINCOLN (University of Maryland)[2] reports that basic lead acetate has been more satisfactory than neutral lead for clearing wood extracts. Basic lead, properly handled, can be used for solutions which do not contain fructose or inulin, but the safety margin is narrow. LOOMIS (**9**) has shown that the more complete clearing with basic lead does not affect the purity of the copper precipitate, although LINCOLN reports better crystallization. The use of basic lead, neutral lead, or no clearing can be justified for different materials. The committee recommends the use of neutral lead clearing as a safe procedure, other methods to be used only after testing.

DELEADING.—The last revision of the A.O.A.C. methods (**1**) requires the anhydrous sodium or potassium oxalate for deleading solutions made to volume before filtering out the lead precipitate. The water contained in crystallized potassium oxalate introduces a dilution error under these conditions. The objection of ENGLIS and TSANG (**5**) to oxalate deleading is based upon determinations which gave a very heavy lead precipitate, and apparently they did not allow time for the oxalate precipitate to crystallize. LOOMIS (**9**) worked with plant extracts instead of the pure sugar solutions used by ENGLIS and TSANG, and found that reducing sugars were lost in the clearing rather than in the lead oxalate precipitate as reported by the latter workers. Sugars held in the lead oxalate precipitate of ENGLIS and

[1] This report was assembled by W. E. LOOMIS for the committee on chemical methods: Z. I. KERTESZ, W. E. LOOMIS, T. G. PHILLIPS, W. E. TOTTINGHAM, chairman.

[2] Location in parentheses indicates private communication.

TSANG were largely removed by cold water washing, but much of the sugar held in the basic lead precipitate of a leaf extract was recovered only by treating the mass with hydrogen sulphide. These results offer no assurance that reducing sugar losses due to the use of basic lead clearing can be overcome by the proper choice of deleading reagent.

SUGAR DETERMINATIONS.—An increasing number of plant chemists are using the SHAFFER and HARTMANN (**14**) sugar method. CLEMENTS (**4**) reports that it has proved more reliable on pea plant extracts than the MUNSON and WALKER (**12**) method. MARSH and DICKSON (**11**) have compared the two methods for sugar determinations on wheat and soy beans. They found the SHAFFER and HARTMANN method to give uniformly higher results, the difference varying with the plant from 5 or 10 to as much as 43 per cent. and becoming less as the plants aged. The percentage deviation was also less for total than for reducing sugars. They state, "It seems possible that some substance which affects the accuracy of the SHAFFER-HARTMANN method is removed or changed by the added acids or by their salts. Also such a material appears to vary with the age of the plant. . . . Further work along this line might reveal that the addition of certain salts would increase the accuracy of the SHAFFER-HARTMANN procedure for use in plant analysis." Poor end points were also encountered in some solutions with the SHAFFER-HARTMANN method.

The convenience of the SHAFFER-HARTMANN method recommends its use whenever possible and it is to be hoped that means may be found to increase the general reliability of the method. It would seem at present that the determination of reduced copper after filtering and washing is still the safest method for general use on plant tissues.

The BERTRAND method (**2**) of determining reducing power is commonly used in Europe, and the recent publication (**8**) of complete tables for use with the method adds to its usefulness. Many American workers prefer to follow the MUNSON-WALKER reducing conditions for which copper-sugar tables are generally available, and to use the BERTRAND method of determining copper by dissolving the cuprous oxide in acid ferric sulphate or ferric alum and titrating the reduced iron with potassium permanganate. Directions for the copper determinations may be found in KERTESZ'S article (**8**) or in the methods section of MATHEWS' Physiological Chemistry.

HYDROLYSIS OF SUCROSE.—WEBSTER (**15**) has reported difficulties with the invertase method and suggests the use of citric acid inversion for sucrose. At the Iowa State College laboratories an invertase method supplied by KRAYBILL has been used in class and research work by some 20 graduate students with complete satisfaction. Four 50-ml. portions of the cleared and deleaded sugar extract are pipetted into 400-ml. beakers. Two of the samples are used for the duplicate determination of reducing sugars.

One or two drops of methyl red solution are added to the other samples, then two to four drops of 10 per cent. acetic acid to bring to the acid color of methyl red, and two to four drops of a 1 per cent. solution of Wallerstein invertase scales. The solutions are allowed to stand for 2 to 24 hours, Fehling solution is added and reducing sugars determined. The blanks for the sucrose determinations should receive the same dose of invertase solution as is used for the samples. Two lots of invertase scale have been used and both have given complete inversion of 50 mg. of sucrose in 50 ml. of solution in two hours at 25° C. The 1 per cent. invertase solution has been kept under toluene and on ice for two months. It is probable that some lots of invertase might require larger quantities of the enzyme or more than a 2-hour minimum period. This point should be checked by varying the inversion time with replicate samples.

With such small quantities of enzyme in place of the heavy dosages sometimes recommended, the expense of the method is negligible and difficulties due to the addition of large quantities of colloidal material are avoided. The use of the entire inverted sample without neutralization or transfer is convenient and time saving, and the specificity of the method recommends it for general use.

SEPARATION OF GLUCOSE AND FRUCTOSE.—Most published analyses of plants report sugars as reducing or non-reducing. When invertase is used for inversion it is considered permissible to multiply the increased reduction, calculated as invert sugar, by 0.95 and report as sucrose. The separate estimation of glucose and fructose in the free reducing sugars is also frequently desirable.

PHILLIS and MASON (**13**) have used a modified iodine method for glucose. Glucose was oxidized according to the procedure of HINTON and MACARA (**6**); the solution was then made acid and excess iodine removed with sodium sulphite. Loss of reducing power with the treatment was recorded as glucose, and the reducing power after treatment as fructose.

In the Iowa State College laboratories JACKSON'S (**7**) method for fructose has been found convenient. Fructose is determined by reduction of OSTS' copper carbonate solution for 75 minutes at 55° C., and glucose by difference of fructose and reducing sugars after correcting for the reducing action of the glucose in the fructose determination. Fructose added to plant extracts has been recovered by this method and hydrolyzed sucrose has given the expected 50 per cent. fructose.

DEXTRINS.—LOOMIS (Iowa State College) reports that two dextrin fractions and no starch have been isolated from the leaves of corn. The first fraction is extracted by 10 per cent. cold alcohol and the second by boiling water. The end points of the two extractions are good although continued extraction by either method apparently results in some hydrolysis of

underlying materials. Extractions are conveniently made by suspending the sample of finely ground (200-mesh) residue, previously extracted with 80 per cent. alcohol, in the 10 per cent. alcohol or boiling water, stirring occasionally for 30 minutes, centrifuging, and decanting the extract. Four extractions have been adequate for corn leaf and stalk tissues. The extracts are cleared with neutral lead acetate, brought to volume, filtered, deleaded, and aliquots hydrolyzed with 1 per cent. hydrochloric acid for one hour at 15 pounds' pressure. Hydrolysis in the autoclave under these conditions has been equivalent to refluxing for two and one-half hours with 2 per cent. hydrochloric acid and is much more convenient. Reducing sugars are calculated as glucose and multiplied by 0.90 to obtain dextrins.

STARCH.—LINCOLN (University of Maryland) finds that fine grinding is necessary for effective removal of starch by enzyme action, and the same report is made by LOOMIS (Iowa State College). At the Iowa laboratory all starch and dextrin samples are ground in a ball mill and passed through a 200-mesh sieve.

KERTESZ (New York Agricultural Experiment Station) reemphasizes a point made in the first report of this committee, that total acid hydrolyzable material should not be reported as starch. Enzyme digestion and clearing before acid hydrolysis is recommended. Clearing of the enzyme extract removes soluble hemicelluloses and gums which would otherwise be hydrolyzed and found as starch. Taka-diastase, animal diastase, and saliva are used for starch hydrolysis. The diastatic power of fresh saliva varies greatly and should be checked. It appears to be preferable to collect saliva before, rather than just after meal time. Both taka-diastase and animal diastase tend to give high results because of the presence of enzymes other than amylase.

CARR (3) has published the results of some very interesting experiments indicating the possibility of dividing starch into fractions which are apparently of economic and possibly of physiological importance. CARR finds that 45 per cent. formic acid separates soluble starch, colloidal starch, and cellulose in ground wheat. Since gums are reported as undissolved, the method would appear to have possibilities both in fractioning total starch and in the routine separation of starch and hemicelluloses.

HEMICELLULOSES.—The physiological significance of these materials has not yet been established and for this reason they should not be determined with the starch or dextrin. The acid hydrolysis of hemicelluloses ordinarily does not give an end point, as measured by the production of reducing substances, but instead drops to a constant low value which probably represents the digestion of cellulose. The hydrolysis conditions should be such that this relatively stable portion of the curve is reached at the end point. LOOMIS (Iowa State College) reports that autoclaving the starch and

dextrin free samples with 1 per cent. hydrochloric acid for one hour at 15 pounds gives a good end point for corn leaf material. As already stated, this treatment has been found to be equivalent to two and one-half hours of refluxing with 2 per cent. acid. In using the method, the powder from which starch and dextrins have been removed is transferred to 250-ml. flasks with 100 ml. of water; 2.7 ml. of concentrated (38 per cent.) hydrochloric acid are added, and the samples autoclaved with a small funnel for a reflux. The extract is then filtered into a 250-ml. volumetric flask and the residue washed. The acid is nearly neutralized with NaOH (m.r.), the solution is made to volume, and its reducing power determined. Clearing is not required for this fraction since the common interfering substances are hydrolyzed by the treatment.

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## DETERMINATION OF NITROGEN IN RELATIVELY SIMPLE COMPOUNDS[1]

### Total nitrogen

A. In the absence of nitrates.—Any of the recognized modifications of the Kjeldahl method may be used. The amounts of catalyst and acid required vary with the amount of organic matter to be oxidized. For 50-cc. aliquots of the alcoholic extract of fruit spurs, containing 1–3 mg. N, 15 cc. concentrated $H_2SO_4$ and 5–7 gm. of a mixture of 10 gm. $K_2SO_4$ and 1 gm. $CuSO_4 \cdot 5H_2O$ were adequate. Frothing is likely to be severe as the last of the water or alcohol is driven off. By careful regulation of the flames and occasional shaking it has been possible to make 30 digestions at once without loss. For the final titration the mixed methyl red-methylene blue indicator proposed by Johnson and Green (**8**) is excellent.

B. In the presence of nitrates.—The use of the salicylic acid method devised by Ranker and described in the previous report (**27**) is troublesome because of the necessity of evaporating an exactly neutral portion of the extract to dryness in the Kjeldahl flask before adding the reagents. This difficulty is overcome by the reduced iron method, which was adapted to plant materials by Pucher, Leavenworth, and Vickery (**11**). The reduction may be carried out in either aqueous or alcoholic solution. The conditions for the reduction prescribed by Pucher *et al.* should be followed exactly. Those for the subsequent digestion may be modified somewhat. For 25-cc. aliquots of the alcoholic extract of tomato leaves, containing 1–5 mg. of nitrogen, 25 cc. of concentrated $H_2SO_4$ and 5–7 gm. of the catalyst mentioned above have been found adequate.

Doneen (**4**) has described a micromethod using salicylic acid. The digest is Nesslerized directly in the presence of gum arabic, and the nitrogen is determined colorimetrically. Preliminary evaporation of a portion of the extract is necessary. As approximately 1 mg. of nitrogen is required for a satisfactory determination, there would seem to be little advantage in this regard over the reduced iron method.

### Fractionation of the nitrogen

The particular methods to be adopted depend on the nature of the material, the method of extraction, and the purpose of the study. Some methods that have proved useful are cited below. Choice must be made in each case of those best adapted to the materials concerned.

[1] This report was assembled by T. G. Phillips for the committee on chemical methods: Z. I. Kertesz, W. E. Loomis, T. G. Phillips, W. E. Tottingham, chairman.

The presence of alkaloids or cyanogenetic glucosides complicates matters considerably. VICKERY and his associates (**12, 22, 23, 25**) have developed methods applicable to tobacco, and DAVIDSON and SHIVE (**3**) have studied the distribution of nitrogen in the presence of a cyanogenetic glucoside.

STUART (**16**) has shown that 80 per cent. alcohol does not extract as much soluble non-protein nitrogen from plant parts as water does, and both he and WEBSTER (**26**) have found changes in the distribution of nitrogen on standing in alcoholic solution. It seems possible that 50 per cent. alcohol might prove satisfactory for extraction, and that changes might be avoided by removing the alcohol from the aliquots used for nitrogen distribution as soon as possible after extraction is complete. Further studies of these points are needed.

Semi-micromethods for ammonia, amide, and nitrate nitrogen, applicable in case extracts can be concentrated sufficiently so that 10-cc. portions contain determinable amounts of these forms of nitrogen, were mentioned in the previous report of this committee (**27**). They are described in detail by STUART (**15**). When portions of alcoholic extracts are freed from alcohol and taken up in water, some of the water insoluble material may form an emulsion. In many cases shaking with chloroform and allowing to stand overnight yields a clear water solution. With some materials a flocculating agent is required. Magnesium sulphate as used by STUART is satisfactory. It is not as effective as calcium chloride, but the latter cannot be used in the presence of nitrates.

### AMINO NITROGEN

This is usually determined in the VAN SLYKE microamino apparatus (**18, 19**). The pipette holds a 2-cc. sample but 4 cc. may be used. An improved reaction chamber has been devised by KOCH (**9**). The sample must be free from alcohol, and the portion used should contain at least 0.2 mg. of amino nitrogen. It may be desirable occasionally to use the larger reaction chamber with the micro burette, so that larger samples, up to 10 cc., can be taken. Description of the apparatus and technique and the necessary tables will be found in the papers cited and in MATHEWS' Physiological Chemistry. VAN SLYKE's manometric equipment may be used for measuring the nitrogen liberated (**20**). The equipment and method are described also by PETERS and VAN SLYKE (**10**).

If ammonia is present in the sample in appreciable amounts it must be removed before the determination of amino nitrogen is made. STUART (**16**) has found that other substances, among which the polyhydric phenols are important, interfere with the method by yielding excessive quantities of gas which is measured as nitrogen. These as well as ammonia are removed or

denatured best by vacuum distillation of the sample at 45° with a slight excess of calcium oxide.

### Ammonia nitrogen

There are two points in connection with this determination which should be emphasized.

1. The treatment of the sample for the liberation of ammonia must be mild in order to avoid the formation of ammonia from compounds other than ammonium salts.

2. Some plants contain appreciable quantities of other volatile bases which are carried over with ammonia in the usual methods of aeration or distillation.

For the removal of ammonia from 10 cc. or less of solution in the determination of urea, Van Slyke and Cullen suggested aeration with an equal volume of 52 per cent. $K_2CO_3$. An adaptation of this method to plant extracts was described in the previous report of this committee, and by Stuart (**15**).

For larger volumes Sessions and Shive (**14**) used $Na_2CO_3$ and NaCl with aeration.

Vickery and Pucher (**22**) removed $NH_3$ from tobacco extracts by a short distillation at atmospheric pressure with magnesium oxide. Schlenker (**13**) has found this method to give somewhat excessive results with other materials. If distillation is to be used, it appears safer to carry it out under reduced pressure at 40°–50°, either with magnesium oxide, or as suggested by Van Slyke (**17**) with calcium hydroxide and alcohol. If the ammonia is determined by titration, volatile bases other than ammonia will be included. Schlenker (**13**) has found this error negligible with a number of plants. With others the error may be comparatively large. Vickery and Pucher (**22**) avoid this difficulty by an adaptation of the permutit method of Folin and Bell. The ammonium ions are taken up by permutit from a slightly acid solution. After washing, the ammonia is liberated by sodium hydroxide and determined colorimetrically with Nessler's reagent.

Schlenker has removed ammonia from plant juices by direct treatment with permutit. As some pigments are absorbed also, aeration from the permutit is necessary before the ammonia can be determined colorimetrically. Various combinations of absorption with permutit and either colorimetric or titrimetric determination are possible. If volatile bases other than ammonia are present in appreciable amounts, the permutit colorimetric combination is to be recommended, since Vickery and Pucher (**22**) have found that these bases either are only slightly absorbed by permutit, or in small amounts do not affect Nessler's reagent.

### Amide nitrogen

This is measured as the increase in ammonia nitrogen caused by mild acid hydrolysis. The exact conditions for the hydrolysis have not been standardized.

Hydrochloric acid has been used most frequently, but Vickery and Pucher (**24**) and Chibnall and Miller (**2**) have shown that large errors are caused by this acid if nitrates are present in appreciable amounts. The other phase of this difficulty, that is, the loss of nitrates on boiling with HCl, was noted in the previous report of this committee, and the use of $H_2SO_4$ was suggested if the amide hydrolysis was to be followed by the determination of nitrates.

Until some standardization of the method is achieved, the conditions described in the previous report may be repeated. These were boiling under a reflux condenser for 2.5 hours with 6 per cent. HCl. As nitrates are usually present, it is best to use 10 per cent. $H_2SO_4$ instead of the 6 per cent. HCl, as this furnishes about the same actual acidity. The addition of 0.6 cc. concentrated $H_2SO_4$ to 10 cc. of the solution gives this result. From the total ammonia nitrogen as determined by one of the methods just referred to, the original ammonia nitrogen is subtracted and the difference is considered amide nitrogen.

This method has given satisfactory recovery of known amounts of the amide nitrogen of asparagine in the presence of nitrate, ammonia, amino and peptone nitrogen.

Pucher[2] finds that the amide nitrogen of glutamine is hydrolyzed much more easily than that of asparagine, and that glutamine occurs in plants more generally than had been supposed. It is possible that some of the nitrogen usually determined as ammonia may come from this source.

### Nitrate nitrogen

Three types of methods have been used for carrying out this determination:

(1) Reduction in alkaline solution with Devarda's alloy and determination as ammonia.

(2) Reduction in acid solution with iron and determination as ammonia.

(3) Colorimetric methods.

In addition to the discussion of the use of Devarda's alloy in the previous report, it should be noted that Sessions and Shive (**14**) determine nitrate nitrogen in a sample in which ammonia nitrogen has been determined, by aerating, after making the solution eighth normal with NaOH and adding 1 gm. of Devarda's alloy. The semi-micromethod outlined in

[2] Reported at the meeting of the New England Section of the American Society of Plant Physiologists, Amherst, Massachusetts, May 25–26, 1934.

the previous report has given satisfactory recovery of known amounts of nitrate nitrogen in the presence of ammonia, amide, amino, and peptone nitrogen. Loomis[3] reports the finding of appreciable amounts of nitrate nitrogen by this method in sweet clover roots which were shown by qualitative tests to contain no nitrates.

It would seem probable that the solution left after the determination of amide nitrogen by any of the usual methods could be used for the nitrate determination. It should be transferred to a Kjeldahl flask, enough alkali added to make a final volume of 300 cc. tenth normal with NaOH, and boiled down to a small volume in order to liberate any ammonia that could be set free by the alkali. After dilution to 300 cc. and the addition of 1 gm. of Devarda's alloy, distillation could be carried out in the usual way on a Kjeldahl rack with an efficient scrubber bulb.

VICKERY and PUCHER (**23**) have described the determination of nitrate nitrogen in tobacco following steam distillation from alkaline solution to remove nicotine and other volatile bases. The nitrate nitrogen is reduced to ammonia in acid solution by reduced iron. A blank or comparison run must be made without the iron. The difference in the amount of ammonia nitrogen obtained in the two determinations represents the nitrate nitrogen of the sample. In samples containing only small amounts of volatile base, the method may be used without the preliminary steam distillation.

PUCHER, VICKERY, and WAKEMAN (**12**) recommend the extraction by ether of nitric acid from plant material that has been acidified to pH 0.7 to 0.9 by $H_2SO_4$. Extracts may be treated in this way by concentration and absorption with asbestos before extraction. Nitrate nitrogen is determined in an aliquot of the extract by the reduced iron method, after making alkaline and removing the ether. A comparison run without the reduced iron must be made in this case also.

Several studies have been made recently of the phenol disulphonic acid method for the determination of nitrates in plant juices or extracts. EMMERT (**5**) has developed a somewhat simplified method for use with tomato and lettuce, and HOLTZ and LARSON (**7**) have described one for wheat. FREAR (**6**) has revised the method of GILBERT in the light of the criticism of various workers, and has proposed a method which would appear to be applicable generally to plant juices or aqueous extracts. If alcoholic extracts are used the preliminary clearing with lead acetate recommended by BURRELL and PHILLIPS (**1**) is likely to be necessary. According to EMMERT the use of NaOH instead of $NH_4OH$ for making the phenol disulphonic acid mixture alkaline avoids off colors with small amounts of sugar, and so would make unnecessary the peroxide treatment used by BURRELL and PHILLIPS. The evidence available appears to preclude the use of charcoal as a clearing agent.

[3] Correspondence.

The following items may be added to the brief description in the previous report of methods for the determination of nitrogen in other forms.

VICKERY (**21**) has discussed the basic nitrogen of plant extracts and has shown that no methods that have appeared as yet for the determination of this fraction yield results that can be interpreted definitely in chemical terms.

VICKERY *et al.* (**25**) determined peptide nitrogen in extracts of tobacco leaves by measuring the increase of amino nitrogen resulting from boiling 20 hours with concentrated hydrochloric acid. They note that this method is not reliable in the presence of nitrates. For this reason the determination was made on the residue from which nitric acid had been extracted by ether as described above. Ammonia formed during the hydrolysis was removed by distillation with magnesium oxide before amino nitrogen was determined.

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## BRIEF PAPERS

# FORMATION OF CHLOROPHYLL AND THE BEGINNING OF PHOTOSYNTHESIS[1]

In 1910 IRVING (**4**), using *Vicia faba* and *Hordeum*, came to the conclusion that etiolated leaves do not possess an appreciable power for carrying on the photosynthesis of carbon dioxide until they have been exposed to light sufficiently long so that they are almost fully green. WILLSTÄTTER and STOLL (**5**) came to a contrary conclusion, since they found leaves with but a small portion of their full chlorophyll content capable of carrying on carbon assimilation quite rapidly. BRIGGS (**1**) believed these experimental discrepancies were due chiefly to the inequality in the age of the leaves used and to the variation in the lapse of time from the greening to the measurement of the photosynthetic activity. DASTUR (**2**) thought the discrepancies noted might be due to loss of water or irregularities in the age of the leaves, together with the difficulty of selecting uniform samples.

### Experimental methods

Seeds of *Zea mays* were planted on moist soil in a dark room. The temperature ranged about 26° ± 2° C. At the end of twelve days the unfolded leaves were collected and floated on tap water kept at 24° ± 1° C.

TABLE I

EXPOSURE OF ETIOLATED LEAVES OF *ZEA MAYS*

| TIME OF EXPOSURE | TEMPERATURE | AGE FROM PLANTING | COLOR OF LEAVES TO EYE | EVOLUTION OF OXYGEN |
|---|---|---|---|---|
| *min.* | | | | |
| 0 | 24° ± 1°C. | 12 days | Yellow | Negative |
| 15 | " | " | " | " |
| 30 | " | " | " | " |
| 45 | " | " | " | " |
| 60 | " | " | " | " |
| 75 | " | " | " | " |
| 90 | " | " | " | " |
| 105 | " | " | Yellow-orange | " |
| 120 | " | " | " | " |
| 135 | " | " | Slightly green | Trace |
| 150 | " | " | " | Positive |

[1] Contribution from the C. F. Kettering Foundation for the Study of Chlorophyll and Photosynthesis.

A 100-watt mazda lamp was placed above the water bath at a distance of 18 inches. Samples of the leaves were tested for the evolution of oxygen before exposure to light and every 15 minutes for a period of three hours. Luminescent bacteria were used to detect the presence of oxygen (3). Table I reports a single experiment.

Other experiments with *Zea, Triticum,* and *Avena* showed variations of from 15 to 30 minutes in the time when the first evolution of oxygen could be detected. However, the evolution of oxygen always began at about the same time that the eye could detect the appearance of a green color in the leaf. Acetone extracts of the same crop of *Zea* leaves before exposure to light gave the following absorption spectrum:

| Bands in ether | Shadow | I | Shadow | II | Shadow | E.A. |
|---|---|---|---|---|---|---|
| | 662.0 | 624.5 | 601.5 | 572.4 | 532.5 | 511.0 |
| Intensity, I, II | | | | | | |

The absorption spectrum after acidification of the above solution with dilute HCl was as follows:

| Shadow | Shadow | I | II | III | Shadow | E.A. |
|---|---|---|---|---|---|---|
| 666.0 | 638.0 | 596.1 | 582.9 | 565.3 | 528.8 | 513.5 |
| Intensity, III, II, I | | | | | | |

When etiolated leaves were exposed to the light of a 100-watt bulb at a distance of 8 inches for two minutes, a representative absorption spectrum was as follows:

| Bands in ether | I | II | III | Shadow | IV | E.A. |
|---|---|---|---|---|---|---|
| | 666.3 | 624.5 | 605.2 | 565.0 | 536.3 | 517.1 |
| Intensity, I, IV, III, II | | | | | | |

Formation of the 666.0 band of chlorophyll could be detected easily after 10 seconds' exposure to the light. After exposure of etiolated leaves for 150 minutes a typical chlorophyll absorption spectrum was obtained.

## Discussion and conclusions

It is not easy to draw definite conclusions concerning the beginning of photosynthesis when respiration must be taken into account. It seems fair to conclude, however, that the first traces of the evolution of oxygen indicate the time when an excess is produced within the plant, and that at least the process of photosynthesis is going on when oxygen is evolved.

From the experimental evidence obtained it is clear that the evolution of oxygen may begin very soon after chlorophyll is formed in sufficient amount to render the young etiolated leaf green to the eye, and is not delayed

until the leaf is almost fully green. On the other hand, an examination of the absorption spectrum of extracts of etiolated leaves after exposure of from ten seconds to two minutes shows that some chlorophyll is formed long before the evolution of oxygen begins.

No attempt was made to test for the absorption of carbon dioxide during the experiments.—O. L. INMAN, *Antioch College, Yellow Springs, Ohio.*

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## SALT CONCENTRATION AND REVERSIBILITY OF ICE-FORMATION AS RELATED TO THE HARDINESS OF ALFALFA

In a previous paper (**1**) it was shown that in four varieties of winter wheat, the concentration of soluble electrolytes in the crowns decreased as the winter season approached. The ice formed in the tissues was determined by the calorimetric method and the unfrozen water computed. The concentration of soluble salts in this unfrozen portion of water was shown to decrease greatly as the plants became more hardy.

In the experiment to be described, plants of three varieties of alfalfa were grown to an age of about six months (August 24 to February 7) in the greenhouse in 6-inch pots. The soil was fertilized and inoculated. Approximately ten plants were grown in each pot. The varieties Grimm, Utah Common, and Hairy Peruvian were selected, since these make the characteristic short day growth. The tops of the Grimm plants remained short, those of the Hairy Peruvian were about 1 foot long, and those of the Utah Common were intermediate.

Samples of roots were severed from the tops at the crown. The roots were washed and the hair like roots stripped off as completely as possible. The samples were rinsed quickly in distilled water, cut into pieces about

2 cm. long, and allowed to dry under moist toweling with occasional mixing. Four samples of 20 gm. each were prepared from each variety on each date, in addition to a sample prepared for determination of dry matter. Because of the considerable amount of fine rootlets formed when the plants are grown in the greenhouse, especially on the Grimm variety, it was never felt that the determination of percentage dry matter was perfectly accurate. It seemed impossible to determine precisely when the samples had become superficially dry, since the fine rootlets formed moist compact masses when the main roots, perhaps 3 mm. in diameter, were dry. This experimental difficulty may be responsible for the seemingly aberrant figures appearing in the data.

TABLE I

VALUES OF SPECIFIC CONDUCTIVITY, IN RECIPROCAL OHMS $\times 10^5$ (25°C.), OF THE EXTRACTS FROM 20 GM. OF ALFALFA ROOTS INTO 300 CC. OF WATER. SPECIFIC CONDUCTIVITY FOLLOWING FREEZING ALONE IS INDICATED AS "FREEZING INJURY" WHILE THAT FOLLOWING SUBSEQUENT HEATING IS SHOWN AS "TOTAL SALTS"

| SAMPLE | FROZEN −6°C. | | | | FROZEN −9°C. | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | FREEZING INJURY | | TOTAL SALTS | | FREEZING INJURY | | TOTAL SALTS | | |
| | FROZEN | | FROZEN | | FROZEN | | FROZEN | | |
| | ONCE | TWICE | ONCE | TWICE | ONCE | TWICE | ONCE | TWICE | |
| | No days' hardening | | | | | | | | |
| Grimm | 44.3 | 50.8 | ..... | 75.0 | 58.2 | 61.0 | 74.2 | 73.8 | |
| Utah Common | 50.5 | 50.0 | 71.0 | 71.0 | 59.2 | 61.0 | 71.0 | 71.0 | |
| Hairy Peruvian | 53.4 | 55.2 | 72.5 | 69.4 | 59.2 | 63.4 | 70.2 | 69.4 | |
| | 10 days' hardening | | | | | | | | |
| Grimm | 34.9 | 32.7 | 74.2 | 74.7 | 48.3 | 55.7 | 74.2 | 75.0 | |
| Utah Common | 42.2 | 42.2 | 71.7 | 70.2 | 54.7 | 57.2 | 70.2 | 69.4 | |
| Hairy Peruvian | 43.3 | 55.2 | 71.0 | 71.0 | 59.2 | 61.0 | 70.2 | 71.7 | |
| | 20 days' hardening | | | | | | | | |
| | | | | | | | | | AVERAGE TOTAL SALTS |
| Grimm | 34.5 | 35.7 | 71.7 | 75.0 | 56.5 | 58.2 | 72.5 | 74.2 | 74.1 |
| Utah Common | 42.2 | 47.2 | 68.7 | 71.0 | 58.7 | 61.5 | 70.6 | 71.7 | 70.6 |
| Hairy Peruvian | 52.0 | 56.1 | 70.6 | 70.6 | 62.0 | 67.2 | 69.8 | 73.3 | 70.8 |

The procedure followed is described in detail in a recent paper (1). The samples were wrapped in tinfoil, placed in tapered, tubular copper containers, and stored at 2° C. overnight to insure a definite temperature at the beginning of freezing. They were frozen by immersion in an alcohol-ice bath for two hours, at −6° C. or −9° C. The duplicate in each case was thawed for two hours at 2° C. and refrozen in the original way. The quantity of ice was determined in each sample immediately on withdrawal from the freezing bath, using the calorimetric method. Injury was estimated from the exosmosis of electrolytes into the water (300 cc.) used in the calorimeter, after a period of 22 hours at 2° C. (2, 3). The samples were then heated to boiling and exosmosis continued for 24 hours, when the total extracted electrolytes were determined electrometrically.

Three series of determinations were made. The first was made on roots of plants direct from the greenhouse. The rest of the pots were placed at

## TABLE II

DATA OF ICE-FORMATION COMPUTED FROM CALORIMETRIC MEASUREMENTS. THE "SALTS PER GM. UNFROZEN WATER" IS COMPUTED BY DIVIDING THE "AVERAGE TOTAL SALTS" BY THE VALUE FOR "GRAMS UNFROZEN WATER" FOR EACH SAMPLE IN TURN

| Sample | Frozen −6°C. | | | | Frozen −9°C. | | | | Percentage dry matter |
|---|---|---|---|---|---|---|---|---|---|
| | Grams unfrozen water | | Salts per gm. unfrozen water | | Grams unfrozen water | | Salts per gm. unfrozen water | | |
| | Frozen | | Frozen | | Frozen | | Frozen | | |
| | Once | Twice | Once | Twice | Once | Twice | Once | Twice | |
| | No days' hardening | | | | | | | | |
| | *gm.* | *gm.* | *gm.* | *gm.* | *gm.* | *gm.* | *gm.* | *gm.* | % |
| Grimm | 5.39 | 4.15 | 13.75 | 17.85 | 3.28 | 3.29 | 22.5 | 22.6 | 42.2 |
| Utah Common | 5.39 | 5.39 | 13.1 | 13.1 | 3.73 | 3.73 | 18.9 | 18.9 | 42.2 |
| Hairy Peruvian | 6.32 | 4.91 | 11.2 | 14.5 | 4.25 | 3.38 | 16.7 | 20.9 | 35.5 |
| | 10 days' hardening | | | | | | | | |
| Grimm | 6.82 | 5.77 | 10.9 | 12.85 | 5.04 | 2.91 | 14.7 | 25.5 | 39.2 |
| Utah Common | 6.01 | 5.58 | 10.8 | 12.65 | 3.73 | 3.88 | 18.9 | 18.2 | 41.6 |
| Hairy Peruvian | 7.27 | 5.04 | 9.73 | 14.1 | 4.04 | 3.54 | 17.5 | 20.0 | 35.9 |
| | 20 days' hardening | | | | | | | | |
| Grimm | 7.01 | 6.36 | 10.6 | 11.65 | 4.12 | 3.72 | 18.0 | 19.9 | 38.7 |
| Utah Common | 6.72 | 5.98 | 10.5 | 11.8 | 4.20 | 3.45 | 16.8 | 21.2 | 40.3 |
| Hairy Peruvian | 4.16 | 3.94 | 17.1 | 18.0 | 2.55 | 0.75 | 27.8 | 94.5 | 40.7 |

a temperature of 2° C. with continuous illumination with mazda lamps. After 10- and after 20-day periods at this temperature, further samples were taken. Tables I and II summarize the data for the experiment.

It will be observed that in the varieties which hardened most, less water was frozen and more left unfrozen when the plants were in the hardy condition than when they were tender. The percentage dry matter in the samples of the hardier varieties tended to decrease slightly during the period in the cold room. These were already "short day" plants, low in vegetative vigor and high in dry matter. This behavior is in sharp distinction from that of winter wheat under the same conditions, where percentage dry matter increases markedly. In the case of the samples of alfalfa, there appears to be no loss of soluble electrolytes during the period in the cold room. This, too, is sharply different from the behavior of winter wheat. Since, however, there is more water left unfrozen when the plants are in the hardy condition than when they are tender, the concentration of electrolytes in the unfrozen sap is less in the hardened plants. This was true to a more marked degree in the case of winter wheat, which also hardens more than alfalfa and will survive lower temperatures.

The data are by no means so clear-cut as those with the winter wheat. The evidence seems conclusive, however, that there is no loss of electrolytes in the case of any of the varieties of alfalfa during hardening itself. It would be worth while to repeat this experiment with alfalfa plants from the field throughout the autumn and early winter period, as was done with winter wheat. Under such conditions the roots are relatively free from the troublesome hairlike roots.

These data, inadequate though they are, are presented at this time since no opportunity to repeat the experiment seems available.—S. T. DEXTER,[1] *University Farm, St. Paul, Minnesota.*

## LITERATURE CITED

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2. ————, TOTTINGHAM, W. E., and GRABER, L. F. Preliminary results in measuring the hardiness of plants. Plant Physiol. 5: 215–223. 1930.
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[1] National Research Council Fellow.

## NOTES

**Summer Meeting.**—The summer meeting of the American Society of Plant Physiologists will be held in Minneapolis and St. Paul from June 24–29. Headquarters for the meeting are at the Plant Pathology and Botany Building, University Farm. The program is being arranged by Dr. R. H. LANDON, Chairman of the Minnesota Section, and RALPH W. LORENZ, Secretary, Division of Forestry, University Farm, St. Paul. The tentative program has been arranged as follows:

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| Monday A. M. | Registration at the A. A. A. S. headquarters. |
| Tuesday A. M. | Joint session, Section O of the A. A. A. S., and symposium on "Improving the germplasm of domestic plants and animals," with addresses by Dean W. C. COFFEY, Hon. H. A. WALLACE, J. L. LUSH, and OLAF AAMODT. |
| Tuesday P. M. | Discussions and inspection of laboratories and demonstration of research work in progress in the physiological laboratory, Department of Botany (Minneapolis Campus) and Section of Plant Physiology (University Farm); also laboratories of the Shelter Belt Project of the Lake States Forest Experiment Station at University Farm. |
| Tuesday evening | A picnic supper on the St. Croix river has been arranged by DR. and MRS. R. B. HARVEY. |
| Wednesday A. M. | Session for presentation of papers of the A. S. P. P., Room 102 Horticulture Building, University Farm. Symposium for presentation of papers on dormancy, after-ripening, and germination of seeds. |
| Wednesday P. M. | Continuation of morning symposium if necessary, with the remainder of the afternoon for papers of general interest. |
| Thursday A. M. | Field trip with plant pathologists and horticulturists to Coon Creek peat experimental plots, commercial vegetable growers (peat soil), early potato section at Osseo, market garden area at Brooklyn Center. |
| Thursday P. M. | Field trip continued to Minnesota Fruit Breeding Farm (accompanied by plant pathologists and horticulturists). |
| Friday, Saturday | A field trip to Itasca State Park, the Cloquet Forest Experiment Station, and North Shore of Lake Superior, joint with Plant Science Group. |

Titles of papers for the symposium on dormancy, after ripening, and germination of seeds, and also papers of general interest, should be sent to RALPH W. LORENZ, Secretary of Minnesota Section, Division of Forestry, University Farm, St. Paul, Minnesota.

The Lowry Hotel in St. Paul has given proper assurance that out-of-town guests will be given every consideration and adequate accommodations if they wish to make it their lodging headquarters. Please make reservations early.

**St. Louis Meeting.**—The twelfth annual meeting of the American Society of Plant Physiologists will be held late in December, 1935, at St. Louis. It is not too early to begin planning for this important series of meetings. Members of the Society can aid the program committee and other officers responsible for the arrangements by prompt response to calls for service. If symposia are contemplated early decisions as to topics and invitation papers would be desirable in order to permit more deliberate preparation by participants. The quality of our meetings can be much improved by careful planning and choice of material. Programs frequently list too many unrelated papers, and so many papers are offered that the program committee should no longer feel obliged to accommodate all offerings. Each meeting affords an opportunity to get out of ruts or fixed grooves and to improve the technique of meetings in general.

**Minnesota Section.**—The Minnesota Section of the Society has held meetings once each month during the year, with an attendance of 15 to 30 at each meeting. The speakers who have addressed the section during the year and the topics under discussion are as follows:

Dr. J. F. McClendon, Physiology in Japan and China.
Mr. F. B. Chandler, Winter injury in apple trees.
Mr. R. E. Oltman, Research on chlorophyll and photosynthesis.
Dr. G. O. Burr, Current physiological research in Europe.

The officers of the Minnesota Section for 1935 are Dr. R. H. Landon, chairman, and Mr. R. W. Lorenz, secretary. Dr. Landon and Dr. Harvey are also taking care of the program and entertainment arrangements for the summer meetings. Those who several years ago attended a previous summer meeting of the Society under the auspices of the Minnesota Section will surely anticipate with much pleasure the June meetings.

**Editorial Committee.**—As was announced in January, the editorial committee is to be reorganized. The executive committee has now arranged the procedure for rotational retirement and annual appointment of memmers of the editorial board of Plant Physiology. The present members of the board have been appointed with retirement dates as follows: C. A. Shull, editor-in-chief, 1939; B. E. Livingston, 1938; F. E. Lloyd, 1937; C. B. Lipman, 1936; C. R. Ball, 1935. As each member retires, a successor will be appointed for a 5-year term. This arrangement provides a degree

of permanence and stability, but permits a slow change in personnel if the executive committee thinks it desirable. The addition of elective members chosen by the society at large waits upon constitutional authority to make such enlargement of the board.

**Finance Committee.**—The finance committee has been placed upon a rotational basis of appointment. As now constituted the finance committee consists of three members: C. A. SHULL, chairman, appointed for 3 years; A. E. MURNEEK, 2 years; W. F. LOEHWING, 1 year.

**International Botanical Congress Delegate.**—The American Society of Plant Physiologists will be officially represented at the sixth International Botanical Congress at Amsterdam in September, 1935, by Dr. WALTER F. LOEHWING, University of Iowa.

**Chemical Methods Committee.**—The committee on chemical methods has been enlarged by the appointment of Dr. H. R. KRAYBILL, Purdue University, to membership on the committee. The committee will continue to serve as a clearing house of information concerning methods of analysis most useful to plant physiologists.

**Chemical Methods Committee Report.**—The first supplementary report of the committee on chemical methods appears in this number of PLANT PHYSIOLOGY. The chairman of the committee, Dr. W. E. TOTTINGHAM, Department of Agricultural Chemistry, University of Wisconsin, Madison, Wisconsin, will have a supply of the reprints of this report bound together as a single reprint. These will be available at a price to be determined by their cost. Announcement of the price will be made in the July number of PLANT PHYSIOLOGY. When it is possible to do so, members should send in club orders for the number needed locally by members and students. This will materially decrease the cost of distribution.

**International Address List.**—A third revised edition of the international address list of plant physiologists has been authorized by the executive committee. It is to be published as Bulletin no. 9 of the American Society of Plant Physiologists, and it is hoped it will be ready for distribution to the members this fall. This address list has been issued at about 5-year intervals, and is a very useful bulletin. As in the case of the first two editions, this one will be compiled and edited by Dr. R. B. HARVEY, University of Minnesota.

**Low Temperature Relations Bibliography.**—For many years Dr. R. B. HARVEY has been engaged in compiling a fairly complete list of the publications dealing with the low temperature relations of plants. This list has now been mimeoprinted by the Burgess Publishing Co., Minneapolis,

with the title "An annotated bibliography of the low temperature relations of plants." There are 195 pages of citations, two columns to the page, and a listing of more than 3,400 items. There are very brief annotations when the titles fail to indicate the nature of the contents of the papers. A subject index occupies 28 pages.

The bibliography represents a very large and unselfish expenditure of time and energy. It will be a most useful work to those who are engaged in investigating any problem involving the effects of low temperatures on plant behavior. As science becomes more complex, and scientists more numerous and more prolific, such bibliographies become more and more necessary to progress in research. The author of this work and his aids deserve praise for having made available this valuable bibliography. The price of the volume in flexible binding is $4, and orders may be sent direct to the publishers.

**Biochemical Laboratory Methods.**—A second edition of Dr. C. A. MORROW's Biochemical Laboratory Methods has been prepared by Dr. W. M. SANDSTROM. The chapters have been reorganized and modified to fit the work to serve as a laboratory manual to go along with GORTNER's Outlines of Biochemistry. The book is somewhat reduced in size, from 350 to 319 pages, owing to omission of some material more adequately supplied in the Outlines. It is a very helpful laboratory manual. The publishers, John Wiley and Sons, quote the price as $3.75 per copy.